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PREFACE

With this volume the *Annual Review of Physiology* completes its second decade. Established to provide a "comprehensive survey of the year's research in physiology," to quote the first preface, it was intended to be "more restrictive than inclusive," to be valuable "not because of its depth of penetration into stated subjects, but because of its breadth." In the face of the enormous increase in publication of physiological literature in the two decades of the Review's life, it may be of interest to see how its authors have reacted to the problem of providing the "comprehensive survey" which the Review has always attempted to provide.

In this period the total number of articles of biological research interest has increased from about 77,000 to 145,000 per year, an increase of 88.3 per cent. In our first volume 4256 papers were reviewed, in the nineteenth, 5543. Assuming the proportion of papers assignable to physiology remained constant, it may be calculated that we took some notice of about 31 per cent less of the physiological literature in the nineteenth as compared with the first volume. In other words, we have become more "restrictive" in our coverage to this degree.

The size of the volumes has increased but slightly—from 260,800 words in 1938 to 264,500 words in 1956. With the increase of papers noticed from 4256 to 5543, the average number of words devoted to an article chosen for review has fallen from 62 to 48. We may thus justly claim to have reduced "the depth of penetration into stated subjects."

Whether we can profitably continue indefinitely our present policies of skimming off one volumeful of physiological cream is a problem with which the Editorial Committee is faced. We invite comment from our readers.

Again we wish to express our deepest gratitude to the men and women who prepared the chapters which constitute this volume; and to rejoice in the fact that all promised chapters reached completion. We wish also to acknowledge the devoted service of the retiring Chairman of our Editorial Committee, Dr. J. P. Baumberger, who will be succeeded in this position by Dr. John Field. Dr. H. W. Magoun is also retiring. The new members will be Dr. J. M. Brookhart and Dr. H. Davenport. We are deeply indebted also to our Editorial Assistant, Mrs. Tove Hunchar, and her co-workers in the Editorial Office for their loyal services, and to our printer, the George Banta Co., Inc. for their indispensable co-operation.

J.M.B.	A.C.G.
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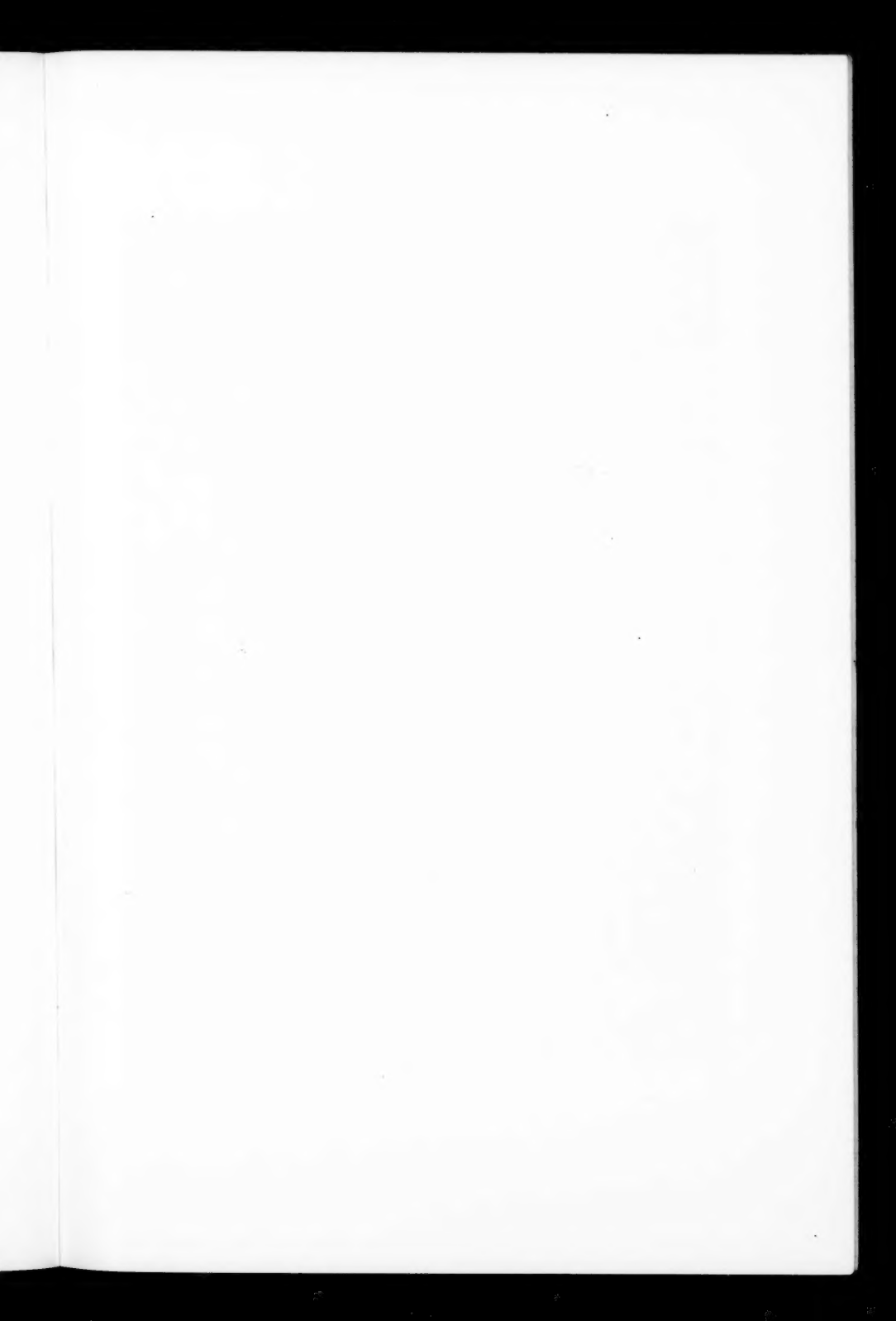
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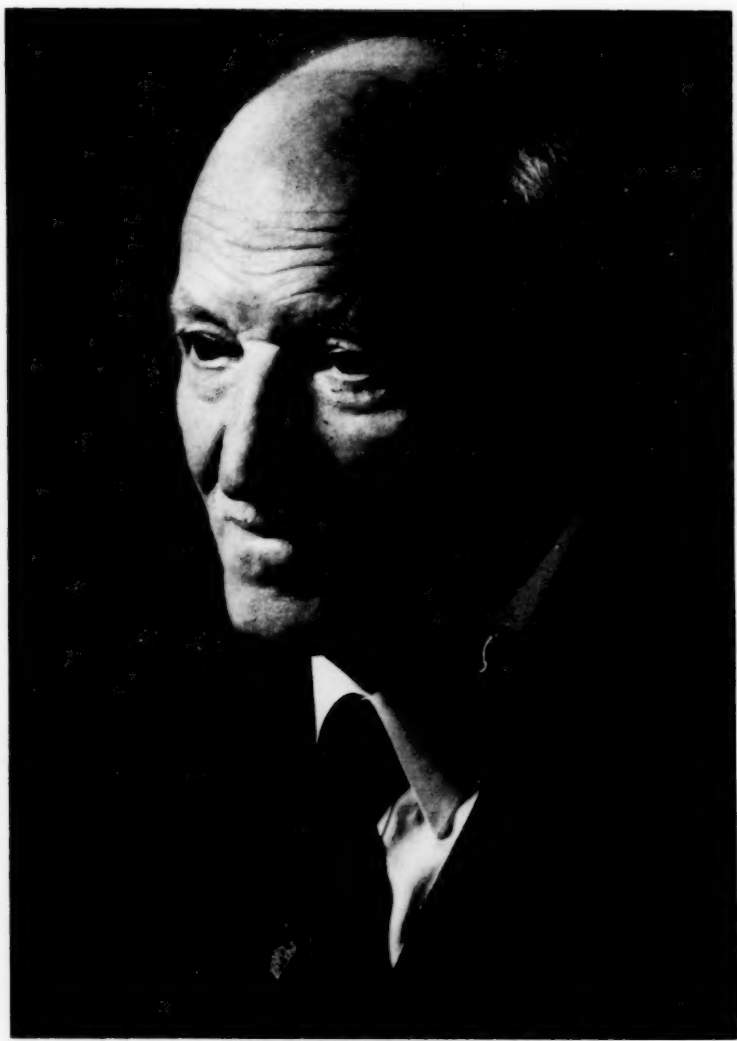
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W. J. V. OSTERHOUT

PREFATORY CHAPTER
STUDIES OF SOME FUNDAMENTAL PROBLEMS
BY THE USE OF AQUATIC ORGANISMS

BY W. J. V. OSTERHOUT

Rockefeller Institute for Medical Research, New York, N. Y.

The writer (1) desires to thank the editor of this series for the opportunity to present a brief summary of some experiments carried out in his laboratory on various aquatic organisms.

These organisms offer special facilities for the study of certain fundamental problems in physiology. The writer was assisted in some of his experiments by W. C. Cooper, E. B. Damon, M. J. Dorcas, E. S. Harris, S. E. Hill, A. G. Jacques, S. E. Kamerling, J. W. Murray, and W. M. Stanley. L. R. Blinks and M. Irwin worked independently in the writer's laboratory.

In 1892 while still an undergraduate at Brown University the writer attended a summer session at the Marine Biological Laboratory in Woods Hole, Mass. In studying the morphology of the marine alga *Agardhiella* [the plant, at that time, was known as *Rhabdonia tenera* (2)] he became interested in the behavior of the asexual spores each of which can give rise to a separate plant. It was found that four spores each capable of producing a new plant could combine to form a single plant. This is the converse (3) of the experiment in which an animal embryo in the four cell stage is shaken so as to separate the blastomeres, each of which then produces a complete embryo. In both cases the original pattern laid down in the germ plasm must be modified.

After joining the faculty of the University of California in 1896 the writer studied the behavior of several species of algae growing on the bottom of steamboats passing regularly from fresh to salt water and back again. When the steamboats were in salt water the cargo was unloaded shifting the water line and exposing the algae to the sun so that salt crystals were formed. In spite of these great changes in osmotic pressure the algae were able to grow (4). In later years experiments were made at Woods Hole on eggs of the marine worm *Nereis*. Unfertilized eggs placed in 1.4 M $MgSO_4$ or in 1.5 M dextrose in sea water lost water rapidly and appeared collapsed with abnormal shapes. If after a few minutes they were replaced in sea water, they soon became normal in size and in appearance. If sperm was promptly added many eggs extruded jelly and segmented (5). These results are in marked contrast to the behavior of many organisms which are injured or killed by relatively small changes in osmotic pressure.

When Jacques Loeb came to the University of California bringing a lively interest in the antagonistic effect of certain ions on animals (6) it was natural to test this on plants. An extensive series of experiments gave very striking

results (7). It was found that NaCl was toxic when it alone was present and this also was the case with CaCl_2 , but when these salts were mixed together in the proper proportions the toxicity disappeared. The effects of incomplete antagonism were studied and certain general conclusions were formulated. Experiments were made to determine the effects of antagonism on permeability and antagonism was discussed in relation to Weber's Law (8). This work on a quantitative basis was made when the writer joined the faculty of Harvard University in 1909.

It became increasingly evident that there was a need for large cells whose sap could be obtained without contamination and which could be studied by placing two or more electrodes on the surface as in the fresh water plant, *Nitella flexilis*, or by inserting a capillary electrode into the vacuole of the cell as in the marine alga, *Valonia macrophysa*. The writer is indebted to the Carnegie Institution of Washington for aid in beginning the study of the former plant at Cambridge, Mass., and to the Rockefeller Institute for Medical Research for the establishment of a laboratory in Bermuda to study marine algae as a result of a grant followed by a staff appointment.

In both these forms (hereafter referred to as *Nitella* and *Valonia* respectively), the protoplasm forms a thin layer surrounding a large central vacuole filled with sap which differs from the external medium.

The sap of *Nitella* contains 0.13 *M* halides (9), 0.05 *M* KCl and 0.05 *M* NaCl (10, 11) but the external medium contains only a small amount of these substances. The pH value of the sap is about 5.5 and the pH value of the external medium is about 6.5 (12).

The sap of *Valonia* differs from the surrounding sea water, a circumstance showing a remarkable protoplasmic selectivity. We find in the sap only a trace of SO_4 although it occurs in sea water at a much higher concentration but the concentration of K^+ inside becomes about forty times greater than that found in sea water (13). The concentration of nitrate (14) inside may be at least two thousand times the concentration outside. The passage of electrolytes through the protoplasm may be extremely slow as compared with a layer of water of equal thickness. This results from the non-aqueous surface layers of the protoplasm which are only slightly permeable to ions.

The analyses show that various ions may enter and reach a concentration higher inside than outside. To account for this we may assume that they do not enter as ions but combine at the surface of the protoplasm with organic molecules called for convenience, carrier molecules, which diffuse into the cell and are there broken up by metabolism into ions which are unable to diffuse out except very slowly (15). They therefore appear to go against a gradient.

To illustrate this we may construct an artificial cell by interposing a nonaqueous layer (chiefly guaiacol) between a solution of $\text{KOH} + \text{KCl}$, representing the external medium, and a solution of CO_2 representing the

living protoplasm in which CO_2 is constantly produced. We then find that K^+ unites with the guaiacol to form K-guaiacolate which passes through the guaiacol layer and reacts with the CO_2 in the artificial protoplasm to form K_2CO_3 and KHCO_3 . The concentration of K^+ in the artificial protoplasm becomes much higher than in the external solution because K^+ cannot pass out through the guaiacol layer except very slowly. We then find an outwardly directed potential which is presumably attributable to the fact that when free ions of K^+ pass outward they have a higher mobility in the guaiacol layer than the anions with which they are associated (16, 17, 18). The concentration of potassium in ionic or in molecular form becomes much higher inside than outside and, if in the external solution K^+ and Na^+ are present in equal concentrations, K^+ enters more rapidly as happens in the living cell (16).

We may regard the guaiacol molecule as a carrier molecule which picks up K^+ at the outer surface and carries it across the nonaqueous surface layer of the artificial protoplasm which is only slightly permeable to free ions. At the same time that combined ions thus move inward some free ions may move outward as shown by the existence of an outwardly directed diffusion potential in certain cells (19) and in models (17). But this movement is very small in comparison with the inward movement of ions in combination with carrier molecules.

It may be noted that the Donnan principle is sometimes invoked to explain the behavior of certain ions in the living cell but it should be remembered that the Donnan principle applies only when no water is allowed to move (20).

It is important to note that the entrance of uncharged molecules is very much more rapid than the entrance of charged ions. This was strikingly shown in the experiments of Irwin (21) on the entrance of brilliant cresyl blue into *Nitella* and later in experiments by other workers (22) with other substances penetrating marine cells. Irwin (21) has shown that the dye penetrates more rapidly at higher pH values in the form of undissociated molecules and dissociates in the acid sap. The process obeys the laws of a reversible pseudomolecular reaction and has a high temperature coefficient.

The nonaqueous layers at the surface of the protoplasm are immiscible with water but can take up a good deal of water and may be quite permeable to it. In *Nitella* their permeability to water may be more than eighteen times greater than that to ethyl alcohol (23).

During stimulation of the cell the permeability of the nonaqueous surface layers of the protoplasm to ions greatly increases as shown by the increase of electrical conductivity. This lasts but a short time but a similar effect lasting a long time may be produced by injury. A simple way to detect injury is to place the cell in a solution of acid fuchsin at pH 7 (19) which penetrates with extreme slowness under normal conditions but enters rapidly when injury occurs. Injury is not necessarily followed by death. When the

action of the injurious agent is stopped in time various degrees of recovery are possible. This subject is of great theoretical interest and is also of practical importance, since it lies at the basis of the art of healing.

It is interesting to find that when a toxic agent is applied at one end of a cell it may produce comparatively little change in the appearance of the protoplasm at the point of application but if the other end of the cell is in contact with water it may show marked changes in appearance because water enters. Thus if a *Nitella* cell is covered in the middle with mineral oil and is in contact with water at the left end (A) we find that application of 0.3 M acetic acid at the right end (B) causes little change in appearance at (B) but marked alterations occur at the opposite end (A) where the chlorophyll bodies become disorganized and dislocated and death soon follows. In the middle of the cell the appearance remains normal for half an hour or more and protoplasmic motion continues as usual (24).

The conception developed here differs fundamentally from the usual view that the effects of injury spread gradually from the region where the toxic agent is applied to the immediately adjoining regions and thence to more remote places.

Life processes consist very largely of chain reactions so that A reacts to form B and this in turn produces C, according to the scheme $A \rightarrow B \rightarrow C$. If the reaction $A \rightarrow B$ slows down, the concentration of B will fall off and this may be injurious because B is necessary for normal cell function. If the reaction $A \rightarrow B$ is then accelerated, the injury may be repaired and the normal amount of B may be restored. But if, in the meantime, substances have diffused out of the cell to a harmful extent, recovery may be delayed until these substances are replaced.

Recovery also implies the restoration of barriers which in the normal cell prevent reactions between various substances from proceeding beyond a certain point.

To understand the nature of injury and recovery it is necessary to put the subject on a quantitative basis. This can be done by measuring the various degrees of injury and the speed with which recovery occurs. Since injury produces an increase in permeability, we may measure the amount of injury by the decrease in electrical resistance which signifies an increase in the penetration of ions.

Measurements of this kind can easily be carried out on cells of the marine alga *Laminaria* (25). Using solutions having the same resistance as sea water, we made the following experiments. When the cells are removed from sea water and placed in a solution of NaCl the cells are injured and the electrical resistance falls until the death point is reached. The time curve of the loss of resistance is very regular and shows that the process is of the first order, in which the loss of resistance in each unit of time is a fixed percentage of the value of the resistance at the beginning of that unit of time. The loss of resistance thus proceeds in an orderly fashion like the loss of heat in a

body during the process of cooling where in each minute the body loses a fixed percentage of the temperature it had at the beginning of that minute (Newton's law of cooling). This gives us a relatively simple picture of the dynamics of the death process.

Injury may be accompanied by an excess of water in the nonaqueous surface layers due to an excess of NaCl in the external solution which appears to have a hydrating effect since it lessens resistance. If the injury has not gone too far, and we add sufficient CaCl_2 to dehydrate the surface layers to the right degree, recovery can take place (19).

If we replace the cells in sea water before the injury (as measured by the loss of electrical resistance) has proceeded beyond a certain point the cells at first show an abnormally low resistance but in a short time the normal resistance returns. This appears to constitute recovery from injury, since the cells live indefinitely after being returned to sea water at the end of the experiment. To obtain this recovery the injury must not go beyond a certain point.

An increase in resistance is produced by CaCl_2 and this might be attributed to a decrease in the water content of the nonaqueous surface layers. It might be suggested that the rise in resistance in CaCl_2 is due merely to the fact that Ca^{++} is less soluble than Na^+ in the surface layers of the protoplasm. But in that case the rise of resistance would take place at once. We find however that it occurs slowly indicating that the permeability of the surface layers is gradually decreased presumably due to dehydration under the influence of Ca^{++} (19). In NaCl there is a gradual loss of resistance presumably resulting from a gradual increase in the water content of the surface layer. Similar results were obtained with other plants and with frog skin (25).

A quantitative theory can be formulated to account for the resistance of the cells in various mixtures of Na^+ and Ca^{++} if we assume that the resistance depends on a substance M (having a high resistance and containing very little water) formed in the nonaqueous surface layers and that the formation of this substance is catalyzed by Ca^{++} and that its destruction is hastened (25) by Na^+ .

Let us now consider the electrical properties of these cells. The methods used are as follows. In order to measure the potential in *Valonia* a capillary glass tube is inserted into the vacuole; this gives constant readings for a long time after the cell recovers from temporary injury (26, 27, 28).

In order to measure the potential in *Nitella*, a spot, B, at one end of the cell is killed with chloroform (26, 29). From another spot, A, we lead off to B and measure the potential. As long as the potential remains constant we conclude that no injury has spread from B to A and consequently measurements made between B and points beyond A give correct values. For each lot of cells we ascertain what concentration of KCl gives zero potential and use this solution instead of chloroform for a reference point (30, 31). In this case no injury to any part of the cell occurs. The results obtained by these

two methods agree. The resting potential in general is about 97 mv.

Let us now lead off by means of a string wet with 0.001 *M* KCl from a spot A on a *Nitella* cell suspended in air with an outwardly directed potential of 97 mv to a spot B where the potential is zero. An exceedingly minute current flows through the measuring instrument to B. This is sufficient to register in a string galvanometer attached to an amplifier (29).

If we plot the change of potential against the logarithm of the concentration of applied KCl we obtain a nearly straight line having approximately the slope required by the Nernst equation for diffusion potential (31, 32). This potential in *Nitella* depends chiefly on KCl since the mobility of K^+ is so great (30, 31).

If we place 0.001 *M* KCl in contact with a *Nitella* cell and lead off to a killed spot, the current flows to the killed spot. This is the traditional negative current of injury. If 0.001 *M* KCl is replaced by 0.1 *M* KCl which does not injure the cell, the current flows to this spot from the killed spot (33). This is attributed to the high mobility of K^+ .

In water the mobility of K^+ differs only slightly from that of Na^+ but in *Nitella* the mobility of the former may be about forty times as great as that of the latter (30). We find that nitrobenzene (34) acts very much like *Nitella* in this respect.

The mobility of an ion depends largely on its attraction for the surrounding medium which it carries with it as it moves. The mobilities of ions in the nonaqueous protoplasmic surface can be controlled in a remarkable manner by certain organic substances. Thus in the outer protoplasmic surface of *Valonia* the order of mobilities is $K > Cl > Na$ (35), but after the addition of guaiaicol the order becomes $Na > Cl > K$ (36). Hexylresorcinol (37), benzene (38), and aniline (39) have been shown to affect the mobilities of ions in *Valonia*.

In *Nitella* under certain conditions the order is $K > OH > Na$ (30). Here, as in *Valonia*, the order of mobilities in the outer protoplasmic surface does not depend on an electric charge on the surface for if this were the case all cations would be faster than all anions or *vice versa*.

The outer protoplasmic surface of the fresh water plant *Chara* (40) shows no alteration of potential when different concentrations of KCl are applied but such alterations are observed after treatment with guanidine so that in this respect it acts like the outer protoplasmic surface of *Nitella*. The outer protoplasmic surface of *Nitella* shows alterations of potentials when the concentration of applied KCl is varied but after leaching for a long time in distilled water this no longer happens. We can thus make *Nitella* behave like *Chara* and *vice versa* (41).

The order of mobility of cations in the outer protoplasmic surface in *Nitella* is $Rb > K > Na > Li$ which is the same as in water (42). This is explained by assuming that the mobility depends on the size of the ion. For example, since K^+ and Na^+ have the same electrical charge, the density of the

charge is greater in Na^+ because the ion is smaller and the charge is condensed on a smaller surface. It therefore attracts a larger amount of water when it moves and consequently its mobility is less than that of K^+ . This applies to Rb^+ and Li^+ also so that we may say that in general the larger the ion the greater is its mobility (43). Cs^+ forms an exception.

The nonaqueous layers of the protoplasm have a low dielectric constant which favors the clumping together of ions to form complex ions as stated by Kraus (44). These facts indicate that an increase in the size of an ion may increase its mobility and we may therefore expect that if any substance in the nonaqueous surface layer unites with another substance its mobility may increase. If NH_3 or some of its compounds or guanidine (in which several NH_2 groups are attached to a carbon atom) unites with K^+ the mobility of K^+ may be increased. This appears to be the case as shown by the following experiments. When the high mobility of K^+ in the outer protoplasmic surface of *Nitella* has been lost by leaching in distilled water it can be promptly restored by the addition of NH_3 or its compounds (45) or guanidine (46). In the outer protoplasmic surface of the fresh water plant *Chara* K^+ has a low mobility but this is greatly increased when guanidine is added (40).

In leaching *Nitella* apparently there is a loss of a substance or substances (called for convenience R). When distilled water in which *Nitella* cells have been leached for several days is shaken with petroleum ether which is then evaporated to dryness, the residue may be taken up in water and applied to the leached cells. These cells are then restored to their normal state so that they can now be stimulated and show high mobility for K^+ (47, 48). This R may be guanidine or a similar compound. Similar restoration can be brought about by many organic compounds (48). CaCl_2 also brings about restoration, possibly by preventing the escape of some organic substances from the cell (49). Pinching the cell and allowing the sap to escape from the vacuole into the protoplasm also restores (50).

It may be noted that the action of guanidine on leached cells may be imitated in many cases by the application of human blood (51) but this may be due to the fact which has been pointed out by Lorente de N6 (52) that human blood contains guanidine. He has studied the effect of guanidine on frog nerve (53). It seems possible that guanidine may play an important role in determining potentials in certain animal cells.

Various substances are known to affect the behavior of nerve cells and are employed in the treatment of nervous diseases. We know that nerves are affected by a variety of organic substances and it seems possible that a study of their effects on potentials may throw light on the mechanism involved in their action.

With a large plant cell containing several ml. of sap it is a simple matter to extract sap without contamination and use this to surround the cell in which the protoplasm forms a thin layer enclosing a large central vacuole

filled with sap. When this is done the inner and outer surfaces of the protoplasm are in contact with closely similar solutions and, if both surfaces have the same potential but one is outwardly directed and the other is inwardly directed, the total value will be zero. This result is sometimes seen in *Nitella* where a value ranging from zero to 16 mv inwardly directed potential is found (29). In *Valonia* surrounded by its own sap an inwardly directed potential is observed (47) when a capillary electrode is inserted into the vacuole (27, 28, 54). These observations indicate that electrical asymmetry is present in some cells.

Although a *Valonia* cell shows a potential when placed in its own sap it quickly dies (54, 55). This may indicate that the sap does not act as a balanced solution for the external protoplasmic surface. In any case this serves as additional evidence of the difference between the inner and outer protoplasmic surfaces. A similar situation was found in certain flowering plants by S. Prat (56).

Cells of the fresh water plants *Chara* and *Nitella* show some very interesting contrasts. In *Chara* the inner protoplasmic surface shows a large potential due to the presence of considerable K^+ in the vacuole and relatively little in the protoplasm (40).

In *Nitella* certain cells which we may designate as *Nitella A* have a relatively large outwardly directed potential at the inner protoplasmic surface due to the presence of 0.05 M KCl in ionic form in the vacuole and relatively little in the surrounding protoplasm. If we designate the total potential as P we may write $P = P_i + P_o$, where P_i is the potential at the inner protoplasmic surface and P_o is the potential at the outer protoplasmic surface. When we reduce P_o to zero by leaching the cell for several days in distilled water the value of P shows little change. We may therefore conclude that the value of P_o is very small. Hence we find that when all of the potential disappears as the result of stimulation the total loss is about the same as in normal cells and in those where the potential at the outer protoplasmic surface has been removed by leaching in distilled water (57).

In addition to the cells designated as *Nitella A* where the potential at the outer protoplasmic surface is small we find others which we may call *Nitella B* where this potential is larger. When the cell is stimulated the potential disappears at the inner protoplasmic surface but remains at the outer surface (58).

These remarkable facts present a fascinating problem and indicate that the inner and outer surfaces of the protoplasm may have very different properties. It would be highly interesting to ascertain whether these surfaces could be made to act similarly by appropriate treatment.

The protoplasm of different species may show great differences in admitting ions. In *Valonia* the protoplasm admits chiefly K^+ to the sap (13) and in *Halicystis* it admits chiefly Na^+ (59, 60). Similar differences are found

in animal cells, for example in human red cells the predominating ion is K^+ and in those of the cat it is Na^+ (61).

When a *Nitella* cell is stimulated very interesting changes occur. Let us consider a cell covered with a thin film of 0.001 M KCl with an outwardly directed potential of 100 mv. At the left end is a spot A; to the right of this we have B, C, D, E, etc., in succession.

To stimulate the cell we reduce the potential at A to a low value. This can be done electrically or by means of KCl (62) or ethyl alcohol (63) or by reduced temperature (64) so that B discharges into A after which C discharges into B and so on so that a propagated electrical impulse passes along the cell. If a spot D is unable to discharge we should, if our picture of the process is correct, be able to bypass it by leading off with a string moistened with 0.001 M KCl (called a salt bridge) passing from E to C so that E can discharge into C after which F discharges into E and so on thus allowing the impulse to progress further along the cell. This experiment succeeds without difficulty thus showing that the picture of the process of propagation is correct (65). The experiments therefore have considerable theoretical significance.

Since a current passes through the salt bridge it would be interesting to substitute a living nerve for the salt bridge to determine whether the nerve would be stimulated so that an animal cell could be stimulated by the plant cell.

It may be added that by means of two salt bridges an impulse can pass from one cell to another (62).

When a propagated electrical impulse causes a loss of potential at any spot the potential is soon restored. But if the next impulse comes along before recovery is complete the spot may be unable to respond. We then have what may be called a one to two ratio. We may also have a one to three, or one to four ratio and so on. A variety of such ratios is found in *Nitella* and they correspond to similar ratios found in the human heart (66). It would seem that *Nitella* offers favorable material for the study of such phenomena.

The outer surface layer of the protoplasm is normally almost impermeable to free ions but ions enter readily when the cell dies as shown by the increase in the electrical conductivity (25) of the cells. The potential falls to zero and remains permanently in this condition.

When the cell is stimulated the potential falls to zero for a few seconds indicating that the surface layers have become permeable to ions and this condition may last for at least one minute when guaiacol or guanidine is present (67, 68).

It seems possible that the increase in the conductivity results from an increase in the water content of the nonaqueous surface layers since they behave somewhat like guaiacol. Shedlovsky & Uhlig (69) have found that the conductivity of dry guaiacol containing potassium-guaiacolate increases

very rapidly as water is added. This is caused by the dissociation of the potassium-guaiacolate.

It is a striking fact that the effects of stimulation closely resemble those produced by death but differ in lasting only a short time. It is therefore not surprising that overstimulation may produce injurious effects.

It is an inspiring task to trace vital processes back to the physical and chemical reactions on which they depend. When we attempt this we are more apt to find new problems than to solve old ones. It is necessary to simplify our problems as far as possible. For this purpose we must first of all find suitable material. Some of the experiments described here illustrate this and it is hoped that they may be helpful to other workers.

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ANIMAL MITOCHONDRIA^{1,2}

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Known by the cytologist for more than sixty years, mitochondria have become during the last decade an important subject of research within several disciplines of experimental biology. For the morphologist, they presented the ideal test objects in applying electron microscopy to the exploration of cellular ultrastructure; for the biochemist, the replacement of "washed tissue particles" by morphologically well-defined entities implied a decisive step towards a systematic mapping of the multienzyme complex of electron transport and oxidative phosphorylation; and for the physiologist, mitochondria afforded a first opportunity for an experimental study of structure-function relationships and of metabolic control mechanisms on a subcellular level of organization.

An attempt to survey the entire literature on animal mitochondria has been made by the present authors in a monograph completed in 1953 (1). Since that time, more than one thousand new references have accumulated in our files. Among these, fortunately, there are a number of comprehensive reviews covering special fields within mitochondrial research, such as mitochondrial ultrastructure (2, 3), tissue fractionation techniques and enzyme distribution data (4 to 9), the enzymatic mechanisms involved in the Krebs cycle (10, 11), metabolism of fatty acids and lipides (12, 13), electron transport (14 to 16) and oxidative phosphorylation (17 to 21).

Because of limited space the original papers to be reviewed within these topics will be selected, first among those not reviewed previously, and the discussion of enzymatic processes will be focused on what is considered to be of special interest from the viewpoint of the physiological function of mitochondria rather than on purely enzymological aspects. Emphasis will be given to the mechanism of mitochondrial energy metabolism and its relation to the mitochondrial structure.

CYTOLOGY

The static picture.—The ultrastructure of mitochondria became available to study thanks to the fundamental contributions of the Palade (2) and Sjöstrand (3) groups. Further valuable sources of recent information about mitochondrial morphology are found in the two symposium volumes, *The*

¹ The survey of literature pertaining to this review was completed May 1, 1957.

² The following abbreviations are among those used in this chapter: AMP (adenosine-5-phosphate); CoA (coenzyme A); DNA (deoxyribonucleic acid); DPN (diphosphopyridine nucleotide); DPNH (reduced diphosphopyridine nucleotide); RNA (ribonucleic acid); TPN (triphosphopyridine nucleotide); TPNH (reduced triphosphopyridine nucleotide).

Fine Structure of Cells (4) and the *Journal of Biophysical and Biochemical Cytology*, Vol. 2, Part I (1956).

The concept that mitochondria are surrounded by a double membrane, and contain a self-perpetuating internal structure giving rise to an increased inner surface, has been firmly established during the last years and found to be valid for all cells investigated throughout the animal kingdom. Current interest concerns primarily a closer analysis of the internal structures and of their relation to the membrane. In most metazoan tissues these structures consist of a number of more or less regularly oriented, double-layered lamellae of the same architecture as the outer double membrane. In Palade's (2) concept, the osmiophilic layers of the lamellae are continuous at one end with the internal mitochondrial membrane, and represent ridges, *cristae*, of the latter. According to this view, the mitochondrial body contains two chambers, one between the two membranes and another inside the folded inner membrane. A somewhat different opinion is held by Sjöstrand (3), who maintains that the lamellae are closed structures, although tightly attached to the mitochondrial membrane, and forming more or less complete *septae* dividing the intramitochondrial space into a number of compartments. Although in some instances, for example in leukocytes (22, 23), numerous open communications can be seen in the electron micrographs between the light central layer of the lamellae and that of the membrane, in other cases (*cf.* 24) the occurrence of such communications is less frequent. Whether this means that the width of the communicating area varies according to the tissue, or that both types of structure actually exist and reflect different types or states of mitochondrial activity rather than different interpretations of the same electron microscopic picture is a problem of immediate importance not only to the cytologist but also, and more and more, to the biochemist.

That the primary function of the *cristae* is connected with an increase of the internal surface rather than a compartmentalization of the inner chamber is indicated by the fact that in some tissues (25 to 35), and especially in the adrenal cortex (25, 29, 31) and at other sites of steroid secretion (31) the disc-shaped *cristae* are replaced by tubular structures, spheric or slightly oval in cross-sections, and protruding as finger-like invaginations from the inner layer of the bounding membrane. As established in the work of Rudzinska, Porter & Sedar (36, 37, 38), the tubular structure is a consistent phenomenon in Protozoa, and Powers *et al.* (27, 39) have advanced the view that tubules may represent the phylogenetically basic type of intramitochondrial structure. Some support for this hypothesis may be found in the observation of Gansler & Rouiller (24) that in liver mitochondrial regeneration following starvation and refeeding of rats the frequency of tubular structures is increased.

The dynamic picture.—By the application of phase contrast microscopy to time-lapse cinematographic observation of mitochondria in tissue cultures

Frédéric & Chèvremont (40) have opened a new, important field of mitochondrial research. The fascinating moving pictures made by the Belgian group are discussed in a series of papers (41 to 43). The mitochondria of the growing fibroblast culture are in lively movement both in interkinesis and during mitosis. Their size, form, and number undergo steady changes, with fusion of several mitochondria into one, and refragmentation into smaller units, as frequent phenomena. The movements appear to be due partly to active forces, and connected with exchange of material with both the nucleus and with the remainder of the cytoplasm. Communications indicating such exchanges have recently also been observed in electron micrographs (30, 44, 45).

Varied experimental conditions may modify the shape and movements of the mitochondria in a dramatic manner. Moderate concentrations of detergents in the culture medium cause a shortening of the mitochondria and a disappearance of the active movements; higher concentrations lead to a swelling with an eventual rupture. A paralysis of the mitochondrial movements with an accompanying shortening is also obtained with ethyl urethane and a series of other metabolic inhibitors. Anaerobiosis, on the other hand, results in a stretching out of the mitochondria, followed by a break-up into small pieces. An elongation of the mitochondria ensues also upon treatment of the culture with dinitrophenol, accompanied by a temporary stimulation of the mitotic activity. The mitotic inhibitor, trihydroxy-N-methylindol, causes a shortening of the mitochondria and an inhibition of their active movement.

Another mitotic inhibitor with a similar action on the mitochondrial movements, 6-mercaptopurine, has been investigated by Bieseke & Tobioka (46, 47). The effect of this agent on mitosis can be reversed by coenzyme A and it has been shown that the reversal is accompanied by a restoration of the elongate shape and the active movements of the mitochondria. These facts, together with the finding that elongate mitochondria stain more intensely with Janus green B than do short mitochondria, are taken by these authors to indicate that the metabolic activity is high in elongate and low in short mitochondria. It may be hoped that studies of this type soon will be extended to include hormones, a field where the electron microscopist and the biochemist both appear to need help.

Origin and relation to other cytoplasmic structures.—"Self-duplication" has been a rather much misused term in attempts to explain the origin of mitochondria. Lately, for example, Leon & Cook (48) observed isolated mitochondria to break apart under the microscope and termed the phenomenon "self-duplication." As has been pointed out by the present reviewers in a previous connection (1), in order to duplicate itself, the mitochondrion should possess the ability to build up its material by itself. There is no justification as yet to believe that this is the case.

Because of the striking structural similarities between cytoplasmic and

mitochondrial membranes, several investigators paid attention to possible relationships, topographic or functional, of the two systems. Lever (49), supported by electron microscopic studies of ACTH-stimulated rat and hamster adrenal cortex, visualized a mechanism by which mitochondria might arise by a curling and subsequent modification of Golgi membranes. Bernhard & Rouiller (50) found a close topographic relationship between mitochondria and endoplasmic reticulum in livers of rats refed after prolonged starvation, or in the regenerative phases after partial hepatectomy or carbon tetrachloride poisoning. It was suggested that mitochondria might play an important part in the elaboration of the endoplasmic reticulum. Under the same experimental conditions, the appearance in the cytoplasm of an increased number of "microbodies" could be observed, characterized by a single membrane, a finely granular matrix, and average dimensions below those of mitochondria (51). These elements, which were first described by Rhodin (52), are suggested by Bernhard & Rouiller (51) as possible precursors of mitochondria. A detailed account of the data and hypotheses of the French group has recently been presented by Gansler & Rouiller (24). An impressive electron microscopic study of the development of chromosomes, mitochondria and Golgi apparatus during spermatogenesis in *Helix pomatia* has been presented by Grassé *et al.* (52a).

The problem of mitochondrial de- and regeneration has been investigated in a series of careful studies by Dempsey & Wislocki (53 to 56), and discussed in an excellent review by Dempsey (57). The summary of this paper gives in a nutshell the actual view of the cytologist about mitochondria:

A characteristic internal structure, consisting of a double-layered outer wall enclosing a matrix-filled space through which pass double-layered membranous folds, would appear to comprise as satisfactory definition of mitochondria for electron microscopy as their intravital affinity for Janus green affords for light microscopy. Relying for identification this characteristic internal structure, mitochondria appear to be pleomorphic structures which vary in size, shape, complexity, and density. They are labile also in that their number may increase or decrease under controlled conditions. The possibility therefore exists that these organelles are constantly being formed and destroyed, perhaps by their participation in metabolic processes.

The problem of the origin of mitochondria is in an unsatisfactory state. New organelles unquestionably are formed in particular physiological states. The possibility that new bodies are produced by fission of ones already present does not seem adequate. On the other hand, the possible fabrication of new mitochondria out of intracellular membranes, although an attractive hypothesis, has not been adequately substantiated.

Tissue fractionation.—Fractionation by differential centrifugation of tissue homogenates made in sucrose is still the most commonly used method to prepare mitochondria. The original procedure has undergone small adjustments, and the latest variant is described by Hogeboom (58). Further

modifications, which have been proposed during the last years aim at an improvement of the structural quality of the isolated mitochondria and of the homogeneity of the fractions obtained.

de Duve and associates (8, 59 to 65) have developed a new scheme of tissue fractionation, which is based on the use of the total applied centrifugal force, rather than on centrifugal force per time unit, as a sedimentation characteristic of the individual fractions. A thorough enzyme distribution screening with this technique enabled the de Duve group to firmly establish the existence of a special type of cytoplasmic granule in liver, distinct from mitochondria, and characterized by a high content of acid phosphatase, RNAase, DNAase, cathepsin and β -glucuronidase. The name, *lysosomes*, has been proposed for these granules. Some biochemical evidence has been given to indicate that the granules are not homogeneous, but made up of a number of particle types, each containing one of the hydrolytic enzymes and surrounded by a permeability barrier. Novikoff *et al.* (66) have recently investigated a lysosome-rich liver fraction with the electron microscope and recognized a number of "dense bodies," smaller than mitochondria, and bounded by a membrane. The relation of these elements to Rhodin's (52) and Rouiller and associates' (24, 51) "microbodies" is an open question. The findings of the de Duve group have essentially been confirmed and extended by Thomson & Moss (67) and by Kuff *et al.* (68), using a density gradient fractionation technique, and by Viala & Gianetto (69). The entrance of the lysosomes on the cytological stage is welcomed by all those biochemists who felt worried about some investigators' attempts to characterize mitochondria on the basis of acid phosphatase or RNAase content.

Using the latency of ATPase and the electron microscopic structure as tests, Witter *et al.* (70, 71) have investigated the bearing of the sucrose concentration on the integrity of the isolated mitochondria. Preparations made in 0.44 M sucrose adjusted with a trace of citric acid to pH 6.2 proved to be optimal from the structural point of view. Greenfield & Price (72) have found that 11 per cent serum albumin or 20 per cent polyvinylpyrrolidone [the latter originally proposed by Woods (73)] when added to the sucrose prevents the enzyme catalase from being dissociated from the mitochondria. Novikoff (74, 75) has subsequently shown that mitochondria prepared in the presence of polyvinylpyrrolidone are better preserved with respect to structure than those prepared in sucrose alone. In order to replace the polyvinylpyrrolidone by a non-nitrogenous compound, and thus to render it possible to determine mitochondrial protein nitrogen, Birbeck & Reid (76, 77) have worked out a medium consisting of raffinose, dextran, heparin, ethylenediaminetetra-acetate (Versene), and AMP. The advantage of this expensive procedure as compared with those suggested by Witter *et al.* (70) and by Novikoff (75) has not yet been documented. Some special problems related to the preparation of mitochondria from cerebral cortex have been discussed

by Shephard (78). The effect of different fixatives on the submicroscopical structure of isolated mitochondria has been investigated by Glimstedt *et al.* (79).

Ottesen & Weber (80, 81) have made an extensive study of the separation of cytoplasmic particles by density gradient centrifugation. The techniques devised by these authors appear to be suited for application to a great variety of cells, including protozoa and eggs, which may contain a rich pattern of cytoplasmic elements. Anderson (82, 83) has examined some theoretical aspects of tissue homogenization and fractionation, and worked out a high resolution differential centrifugation procedure. An evaluation of the heterogeneity of cytoplasmic particles obtained by differential centrifugation has been made by Johnson *et al.* (84). Novikoff has reviewed and critically examined current tissue fractionation methods (9).

Holter (86) cut centrifuged amoebae into "light" and "heavy" halves and showed that the enzymes succinic dehydrogenase, acid phosphatase, and cathepsin are recovered in the "heavy" halves, containing the cytoplasmic particles. The same enzymes were found in the particulate fraction after centrifugal fractionation of homogenized amoebae. Holter's data are one of the few independent verifications of tissue fractionation.

CHEMICAL COMPOSITION

Earlier data on the chemical composition of mitochondria have been reviewed by Schneider & Hogeboom (6, 87) and by Lindberg & Ernster (1).

Whether or not mitochondria contain RNA is not yet definitely settled. It is generally agreed that the RNA content of mitochondria, if any, must be relatively low. Several authors have attempted to decide the question by comparing the composition of RNA in various subfractions. Olmsted & Villee (88) found three kinds of RNA in adult and fetal human liver, one in the nuclear fraction, another in the soluble cytoplasm and a third which is present in both the mitochondrial and the microsomal fractions. In rat liver, on the contrary, only one kind of RNA was found. These data are at variance to those by de Lamirande *et al.* (89) who concluded that the RNA composition of each of the cell fractions, nuclei, mitochondria, microsomes, and final supernatant, differs from those of the others and is specific for the fraction from which it has been isolated. These authors have also found (90) that treatment of mitochondria with RNAase results in a release of the latent acid phosphatase. However, acid phosphatase is no mitochondrial enzyme (59 to 65). Laird & Barton (91) have investigated the distribution of total protein and RNA among cell fractions in various normal and neoplastic tissues.

According to recent data by Spiro & McKibbin (92) the amount and types of lipides vary from one cell fraction to another. The microsomal fraction is richest in lipides, the mitochondria less rich and the nuclei still less, while the soluble fraction is almost lipide-free. The lipide content of the cytoplasmic particulates is made up in largest part by phospholipides. The

authors have also investigated the effects of choline deficiency on the lipid distribution pattern.

The nucleotide content of mitochondria has been analyzed by Siekevitz & Potter (93). Comparing the mitochondrial nucleotide pattern with that obtained by Hurlbert *et al.* (94) for the whole liver, it is obvious that the number of nucleotides is less in the former case. Especially striking is the virtual lack of uridine and guanine nucleotides in mitochondria. None of the analyzed nucleotides has been found to be concentrated in the mitochondria. Glock & McLean (95) have investigated the intracellular distribution of DPN and TPN in rat liver. The data reveal that about 15 per cent of the cellular DPN and about 50 per cent of the cellular TPN is present in the mitochondria, the former mainly in the oxidized, and the latter almost exclusively in the reduced form. It is noticeable that mitochondria contain more TPN than DPN on the molar basis.

The intracellular distribution of sodium, potassium, calcium, and magnesium ions in rat liver was studied by Grisvold & Pace (96), and that of potassium ions also by Berger (97). Evidence is presented in both papers that the analyzed ions are present in mitochondria in a nondiffusible form. Maynard & Cotzias (98) injected Mn^{56} into rats and found it to concentrate in organs rich in mitochondria. They concluded that mitochondria are the principle site of manganese uptake.

ENERGY METABOLISM

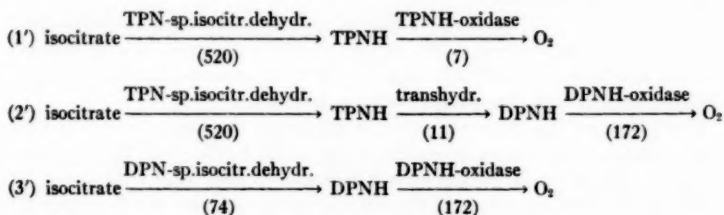
Data bearing on the enzymatic composition of mitochondria, based on enzyme distribution studies, have been reviewed in numerous instances (4 to 8, 87). As an additional recapitulation of these data is considered to be of little interest, the present section will be restricted mainly to some current problems connected with the role of mitochondria in cellular energy metabolism. The problems are of two kinds. One emerges from the fact that although isolated mitochondria are known to be able to catalyze the aerobic oxidation of any member of the Krebs tricarboxylic acid cycle, only cytochrome oxidase and a few additional enzymes involved in this process are exclusively localized in the mitochondria. The question therefore arises as to whether, and to what extent, the mitochondrial operation of the Krebs cycle is dependent on the participation of extramitochondrial enzymes. The same kind of problem concerns the autonomy of mitochondria in fatty acid metabolism.

The second type of problem is that of the relation of the mitochondrial energy transferring system to the mitochondrial structure. In this connection, the mechanisms of DPNH- and TPNH-cytochrome-*c* reductase and of oxidative phosphorylation deserve special interest.

Krebs cycle.—The main difficulty in accepting the early view (99) that mitochondria are the major operational site of the Krebs cycle has been the fact, first pointed out by Hogeboom & Schneider (100), that only about 12 per cent of the TPN-specific isocitric dehydrogenase activity of a liver homog-

enate is recovered in the mitochondrial fraction, the bulk of the enzyme being found in the soluble cytoplasm. The arguments that this low percentage might represent a nonmitochondrial contamination and that, even if this is not the case, it would be insufficient to bring about an adequate rate of isocitrate oxidation along the Krebs cycle, have been repeated for more than six years (4 to 7, 87), without any new experimental data having been added to the discussion. Recently Schneider *et al.* (101) showed that in normal and fluoroacetate-poisoned rats the bulk of the citrate present in liver can be recovered in the mitochondria. This finding was taken to indicate that the mitochondrial membrane is poorly permeable to citrate, and it was employed (5) as a further support for the idea that most of the citrate formed in mitochondria is oxidized in the soluble cytoplasm. If the conclusions of these authors are correct, it is difficult to understand how the Krebs cycle can operate at all in the liver cell.

A somewhat different conclusion has recently been obtained by Ernster & Navazio (102 to 104) in an investigation of the pathways of isocitrate oxidation in rat liver mitochondria. The results of these studies can be summarized as follows: (a) The TPN-specific isocitric dehydrogenase is distributed between mitochondria and soluble cytoplasm, in conformity to Hogeboom & Schneider's data (100), in the approximate proportions 1:9. A DPN-specific isocitric dehydrogenase, an enzyme first described in mitochondria by Plaut & Sung (105), is exclusively located in mitochondria. (b) The TPN-specific enzyme present in mitochondria is truly mitochondrial (and not contamination) as revealed by the fact that its activity, as measured with added TPN as terminal hydrogen acceptor, increases upon aging of the mitochondria. This phenomenon has previously been observed with glutamic dehydrogenase (106, 107) which is an entirely mitochondrial enzyme. (c) In aged mitochondria, depleted of their complement of pyridine nucleotides, added DPN almost completely restores isocitrate oxidation, while added TPN has only a slight effect. This finding was taken to indicate that isocitrate is oxidized mainly by way of the DPN-specific isocitric dehydrogenase. The same finding was independently reported by Kaplan *et al.* (108) who postulated that the pyridine nucleotide transhydrogenase was mainly responsible for the observed DPN-effect. (d) Kinetic studies of the individual reactions revealed that the aerobic oxidation of isocitrate in rat liver mitochondria proceeds along three pathways:



The figures in parentheses indicate the capacities of the individual reactions as percentages of the rate at which isocitrate is oxidized by oxygen in isolated rat liver mitochondria. These data show that the DPN-specific isocitric dehydrogenase is the main pathway of isocitrate oxidation, and, furthermore, that the TPN-specific isocitric dehydrogenase constitutes no limiting reaction in the mitochondrial operation of the Krebs cycle.

Metabolism of fatty acids and lipides.—As has been known since the fundamental studies of Kennedy & Lehninger (109), oxidation of fatty acids is a typical mitochondrial process. The mechanism of oxidation of fatty acids containing an even number of carbon atoms has largely been clarified during the last years, and the enzymes involved have been isolated from mitochondria (12, 13, 110). Recent work of Lardy's laboratory has thrown some light on the mechanism of some odd-numbered and branched fatty acids in liver mitochondrial systems. The reactions studied imply a biotin-dependent carbon dioxide fixation as the initial step. Thus, Fischer (111) showed that β -methyl- C_3 -fatty acids condense with bicarbonate in rat liver mitochondria, with a subsequent cleavage yielding acetoacetate. Lardy *et al.* (112, 113) studied the carboxylation of propionate to succinate in rat liver and found that the enzyme system involved is concentrated in mitochondria.

The individual reactions involved in the fatty acid oxidation cycle have been shown to be reversible and, consequently, the synthesis of fatty acids might be expected to be a mitochondrial process. However, an additional enzyme, responsible for the reduction of the acyl-CoA dehydrogenase flavoprotein, appears to be required for the biosynthesis of fatty acids, and the intracellular localization of this enzyme is not yet known (12, 13). Recent evidence indicates that in liver this reaction requires TPNH as a specific hydrogen donor (114 to 116). As is known from earlier studies of Brady & Gurin (117), for fatty acid synthesis to take place in a cell-free liver system, the presence of both the mitochondrial and the soluble fractions is required. Whether the role of the soluble fraction is to supply the acyl-CoA dehydrogenase reducing enzyme, or to generate free TPNH by way of the isocitric or glucose-6-phosphate dehydrogenase reactions, is not decided as yet. A somewhat different situation from that in liver seems to prevail in the lactating mammary gland, where, as is indicated by the data of Popják & Tietz (118), TPNH is not required, and fatty acid synthesis can proceed in a particle-free, soluble system.

Weiss, Smith & Kennedy (119, 120) succeeded in obtaining synthesis of lecithin from phosphorylcholine and α - β -diglyceride in a rat liver mitochondrial system. The reaction mechanism, which involves cytidyldiphosphate choline as an intermediate, has been found to be concentrated in mitochondria.

The conversion of cholesterol into various acid steroids in mouse liver mitochondria was reported by Fredrickson (121).

DPNH- and TPNH-cytochrome-c reductases.—The concept, originating from Lehninger (122), that DPNH is oxidized in mitochondria by way of

two types of cytochrome-*c* reductase, one connected with phosphorylation and the other not, has become firmly established and widened. The two types of enzyme differ with respect to their cytochrome-*b* component (123 to 126) and in their sensitivity to antimycin A (17, 62, 127 to 129) and amobarbital (Amytal) (129 to 132), which selectively inhibit the phosphorylative type. Antimycin A, which is a well-known inhibitor of the succinic oxidase system (133 to 135), blocks the reoxidation of cytochrome-*b* by cytochrome-*c*, while Amytal, which does not inhibit the oxidation of succinate (130, 132), is supposed to act at the flavoprotein level (132, 136).

While the antimycin A- and Amytal-sensitive type of DPNH-cytochrome-*c* reductase is exclusively mitochondrial, the insensitive type is present in both the mitochondrial and microsomal fractions (cf. 62). The possibility has repeatedly been considered that the presence of the insensitive type of reductase in mitochondrial preparations might be due to microsomal contamination (7, 17). However, recent data by de Duve *et al.* (62) seem to eliminate this possibility. According to these data the same percentage ratio, about 40 per cent, of both the total DPNH- and TPNH-cytochrome-*c* reductase activities present in mitochondria is insensitive to antimycin A. This could not be the case if the antimycin A-insensitive activities were due to microsomal contamination, since the microsomes contain about 73 per cent of the total cellular DPNH-cytochrome-*c* reductase activity, but only about 36 per cent of the total cellular TPNH-cytochrome-*c* reductase activity. Furthermore, the presence of 40 per cent insensitive reductase activity in the mitochondria would imply, on the TPNH-cytochrome-*c* reductase activity basis, a microsomal contamination amounting to more than one-third of the total microsomal fraction, which is very improbable.

In intact mitochondria, the two types of DPNH-cytochrome-*c* reductase are functionally separated. DPNH generated within the mitochondria is mainly oxidized by way of an antimycin A- and Amytal-sensitive type of reductase, whereas DPNH generated outside the mitochondria is preferentially oxidized by the insensitive pathway, provided that an external supply of cytochrome-*c* is present (17, 122, 129, 137). In order to explain this functional separation Lehninger (17) visualized the two types of DPNH-cytochrome-*c* reductase systems as being located inside and outside, respectively, a semipermeable mitochondrial membrane. However, the situation seems to be somewhat more complicated. Thus, as found by Ernster (129), DPNH generated by intramitochondrial dehydrogenases reacts preferentially with the Amytal-sensitive type of DPNH-cytochrome-*c* reductase even after the mitochondria had been exposed to moderate aging or Ca^{++} treatment and thus rendered dependent on added DPN. It was concluded that DPN is released from the mitochondrial structure more readily than is DPNH and that the latter is oxidized by the Amytal-sensitive pathway as long as it is bound. Furthermore, as emerges from recent data by

Colpa-Boonstra & Slater (138), the DPNH-cytochrome-*c* reductase present in Keilin and Hartree heart muscle preparations is completely inhibitable with antimycin A. As these preparations consist, according to Cleland & Slater (139, 140), of fragments of the sacrosomal membranes, it would appear reasonable to assume that the antimycin A-sensitive type of DPNH-cytochrome-*c* reductase is associated with the mitochondrial membrane. On the other hand, the electron microscopically authenticated membrane preparations recently obtained by Siekevitz & Watson (141) from deoxycholate-treated rat liver mitochondria seem to contain no noticeable DPNH-cytochrome-*c* reductase activity of any type. Some further aspects of the alternative pathways of mitochondrial DPNH-oxidation have been discussed by Ernster (104, 142).

Martius (19) has advanced the hypothesis that the phosphorylative pathway of DPNH-cytochrome-*c* reductase may involve the participation of the two fat soluble vitamins K and E, or derivatives thereof. The former would be inserted as an electron transmitter between DPNH and cytochrome-*b*, and the latter between cytochrome-*b* and cytochrome-*c*. There is some support available for the involvement of these vitamins in mitochondrial oxidations (138, 143 to 147), but direct evidence for their role in oxidative phosphorylation in the sense visualized by Martius is still lacking.

Oxidative phosphorylation.—The mechanism of oxidative phosphorylation is one of the most active fields of present biochemical research. The intact mitochondrion has been, and still seems to be, the best suited organization level for this research, and, in fact, a considerable percentage of all experiments hitherto performed with mitochondria have been done for the purpose of studying oxidative phosphorylation.

Considerable progress has been made during the last years in clarifying the number and localization of phosphorylations along the respiratory chain. According to the generally held view, the aerobic oxidation of one mole DPNH to DPN is connected with the esterification of three, and that of succinate to fumarate with the esterification of two moles of orthophosphate; concerning the oxidation of TPNH, the occurrence of coupled phosphorylations is reasonably well established (cf. 103), but their number is not yet settled. Of the three phosphorylations connected with the oxidation of DPNH, one is supposed to occur in the electron path between DPNH and flavin, another between flavin and cytochrome-*c*, and the third between cytochrome-*c* and cytochrome oxidase. The work underlying these concepts was obtained mainly in studies in which phosphate uptake was measured in isolated mitochondrial systems incubated in the presence of various substrates and electron acceptors under carefully controlled conditions; the pertinent literature has been amply reviewed by Lehninger (17).

A valuable verification of these results has recently been achieved by Chance & Williams (18) in a series of highly qualified kinetic studies in which the steady state levels of oxidation-reduction of some intramitochondrial

drial electron transporting catalysts, *viz.* pyridine nucleotides (DPN + TPN), flavins, and individual cytochromes, were recorded spectrophotometrically under varied experimental conditions. These investigations were based on the concept, developed by Lardy & Wellman (148), that in intact mitochondria respiration can be rendered dependent on the availability of a terminal phosphate acceptor. The results so obtained enabled Chance & Williams (18) to postulate that the three phosphorylations coupled to the aerobic oxidation of DPNH are located at the sites, DPN, cytochrome-*b* and cytochrome-*c*, and that the reduced forms of these respiratory catalysts are carriers of the previously formed high-energy bonds. One difficulty in accepting this latter conclusion as correct is that it is based on the assumption that no alternative pathways exist for the mitochondrial oxidation of DPNH. A critical evaluation of Chance & Williams' kinetic studies has recently been presented by Holton (149).

The well-known uncoupling effect of 2,4-dinitrophenol on oxidative phosphorylation, the widely studied phenomenon of the latent mitochondrial ATPase, and the recent discovery of the phosphate-oxygen and ATP-phosphate exchange reactions taking place in mitochondria, form, at present, a promising basis for continued studies of the mechanism of oxidative phosphorylation. Intact mitochondria catalyze the replacement of orthophosphate-oxygen by water-oxygen (150 to 153) and the terminal phosphate group of ATP by orthophosphate (151, 153 to 156) at rates which are independent of, and can be considerably higher than, the net rate of oxidative phosphorylation. Boyer *et al.* (151), who were first to investigate these reactions in the absence of oxidizable substrate, found that both types of exchange reaction are inhibited by dinitrophenol. This indicated that the exchange reactions are constituents of the reaction sequence of oxidative phosphorylation, and that the latter involves at least one high-energy intermediate prior to the formation of the primary high-energy phosphate bond. Furthermore, the findings gave support to the earlier hypotheses (148, 154, 157 to 160) that the dinitrophenol-induced ATPase activity of mitochondria is due to the hydrolysis of a high-energy intermediate in oxidative phosphorylation.

The ATP-phosphate exchange and dinitrophenol-activated ATPase reactions have recently been subjects of a number of inhibitor studies (153, 156, 161 to 168), with the hope of obtaining information about the exact nature of the enzymes and intermediates involved in oxidative phosphorylation. The advantage in using these reactions, rather than net phosphorylation, as test systems lies in the fact that they can be measured in the absence of added oxidizable substrate and are thus suited for studies of the bearing of the oxidation-reduction state of the respiratory chain on oxidative phosphorylation. Several schemes have been proposed (17, 18, 21, 160, 166, 167) to explain the mechanism of coupling between electron transport catalysts and hypothetical compounds to form primary high-energy bonds, but only a few attempts have been reported as yet to verify these by investigating the

effects of anerobiosis or of respiratory inhibitors on the ATP-phosphate exchange or the dinitrophenol-activated ATPase reaction (153, 156, 166, 168, 169).

When mitochondria are exposed to "aging," their ability to exhibit oxidative phosphorylation (93, 157, 170 to 173) and phosphate-oxygen and ATP-phosphate exchange disappears (153) and dinitrophenol-activated ATPase (161, 174) disappears, with the simultaneous appearance of a Mg^{++} -dependent ATPase activity (157, 158, 161, 174, 175). Although it might appear logical to visualize a simple functional relationship between the two phenomena, available data indicate (175 to 180) that the problem of mitochondrial ATPase is a complex one, involving many variables. Yet, it is noticeable that ADP (176), pentachlorophenol (163, 164), azide (165), and *p*-chloromercuribenzoate (158, 162) are inhibitors of both the dinitrophenol- and the Mg^{++} -activated ATPases.

A number of short reports have appeared, accounting for the preparation of various soluble factors, mostly derived from mitochondria, with a marked stimulatory (181 to 185) or inhibitory (186 to 188) effect on oxidative phosphorylation. Attractive in perspectives, the bearing of these findings on the mechanism of oxidative phosphorylation is yet to be assessed.

Submitochondrial systems.—Considerable progress has recently been made towards an elucidation of the intramitochondrial localization of the respiratory enzyme system. Siekevitz & Watson (85, 141) have isolated, from deoxycholate-treated rat liver mitochondria, a particulate preparation which contains succinic oxidase activity in a five- to seven-fold concentration above that in the mitochondria, by following in the electron microscope the stepwise destruction of mitochondria by deoxycholate. This preparation could be identified as a derivative of the mitochondrial membrane system. The conclusion earlier arrived at by Cleland & Slater (139, 140) that succinic oxidase resides in the mitochondrial membrane appears thus to be substantiated. The membrane preparation of Siekevitz & Watson (85, 141) contains essentially no DPNH-cytochrome-*c* reductase and is devoid of ability for oxidative phosphorylation.

Cooper & Lehninger and associates (167, 179, 189 to 194) disrupted mitochondria in a one per cent digitonin solution and thus arrived at a particulate preparation which can carry out oxidative phosphorylation. Succinate and β -hydroxybutyrate were found to be the only substrates to be oxidized, yielding phosphate/oxygen ratios between 1.5 and 2.4. The phosphorylation is sensitive to the known uncouplers, dinitrophenol, Dicumarol, gramicidin and pentachlorophenol, but not to Ca^{++} or thyroxine. Furthermore, in contrast to intact mitochondria, the phosphorylation in these preparations is depressed rather than stabilized by Mg^{++} . The preparation contains bound DPN; added DPNH is oxidized, as in intact mitochondria, by way of a non-phosphorylative type of cytochrome-*c* reductase. The preparation exhibits a Mg^{++} -dependent ATPase activity. The dinitrophenol-activated ATPase and the ATP-phosphate exchange reactions are present but relatively slow.

A comprehensive study of the electron transport system, wearing the marks of Keilin and Hartree's traditions, has been undertaken by Green and his group (195 to 202). A particulate system, prepared from stored, large-scale preparations of beef heart mitochondria, catalyzing the aerobic oxidation of DPNH and succinate, served as starting material for these investigations. By various treatments the Green group succeeded in modifying the catalytic properties of this multienzyme system and isolated single enzymic components, such as succinic dehydrogenase, DPNH-dehydrogenase, the corresponding cytochrome-*c* reductases, and cytochrome oxidase. These components were analyzed for flavins, hemes and nonheme metals, and the molar relationship of these constituents was established. More recently also the successful isolation of a system of phosphorylative electron transport particles has been reported (203 to 205), capable of carrying out oxidative phosphorylation coupled to the oxidation of Krebs cycle intermediates except succinate.

Submitochondrial preparations capable of oxidative phosphorylation have also been described by Raw (181) and by Kielley & Bronk (206).

Relation of biochemical organization to structure.—Since the early observation of Claude (207) that mitochondria swell when placed in a hypotonic medium many attempts have been made to characterize mitochondria as more or less perfect osmometers, obeying the laws of passive permeability [cf. (8, 208)]. Today it appears to be established beyond reasonable doubt that this property of the mitochondrial structure ultimately depends upon an active principle, closely associated with the enzymatic organization of the mitochondrion.

This concept originates from the widely recognized fact that mitochondria incubated in the absence of conditions favoring oxidative phosphorylation take up water, regardless of the tonicity of the medium. The water uptake is revealed by a swelling under the phase contrast microscope (209), a decrease in the optical density of the mitochondrial suspension (210), and a reduction of the relative dry weight of the mitochondrial pellet (211). During this "aging" process, the mitochondria gradually lose the ability to concentrate various ions (211, 212) and nucleotides (93) and to carry out oxidative phosphorylation (93, 157, 171 to 173) and phosphate-oxygen exchange (153). Simultaneously, they acquire certain hydrolytic activities such as DPNase (170), acetyl-CoA deacylase (213) and a Mg^{++} -dependent ATPase (157, 158, 161, 174, 175).

According to an early interpretation (209) of the swelling phenomenon, an active oxidative phosphorylation should be a prerequisite for the maintenance of the structural integrity of the mitochondrion. This view had to be abandoned, however, since it was recognized that the aging process can be influenced, enhanced or delayed, by means of agents which, substrate being absent, cannot be of relevance to oxidative phosphorylation. Ca^{++} (210, 214) and phosphate ions (170) are typical labilizers of the mitochondrial structure, whereas Versene (210, 214), Mg^{++} (170, 210), Mn^{++} (172, 173,

215, 216, 217) and adenine nucleotides (210, 211, 218, 219) are known to protect mitochondria against aging; a comprehensive list of further agents influencing mitochondrial swelling has recently been presented by Tapley (220). Moreover, dinitrophenol (194, 220) and anaerobiosis (171), both of which abolish oxidative phosphorylation, have recently been found to delay, rather than enhance, the aging of mitochondria.

Among the agents which are able to protect mitochondria against the consequences of aging, ATP has received special attention, since it is also capable of restoring, at least to a certain degree, the structural and functional integrity of an already aged mitochondrial system. Thus, ATP can bring about a contraction of the swollen structure (211, 217, 221), accompanied by a resumption of the ability to concentrate ions (211) and nucleotides (129, 222) and to exhibit oxidative phosphorylation (172, 173, 211, 215, 216, 217) and phosphate-oxygen exchange (153). Furthermore, as recently observed by Gamble (223), ATP is also able to prevent mitochondria from aggregation caused by cytochrome-*c*. ATP appears to be specifically required for these reactions; ADP is less efficient, and AMP is without effect (172) except if substrate is present and swelling has not proceeded too far (211).

To explain the protective effect of added ATP on mitochondrial structure, Raaflaub (210) proposed that mitochondrial swelling may be due to a loss of intramitochondrial ATP, the latter being of primary importance for the maintenance of the structural integrity of the mitochondrion. Some experimental support for this idea was obtained by Brenner-Holzach & Raaflaub (224) who showed that the swelling of mitochondria upon aging is closely paralleled by a release of mitochondrial ATP, and Ernster & Löw (172), according to whose data aging of mitochondria in a Mg^{++} -free sucrose-phosphate medium is accelerated by hexokinase and glucose. This latter finding could not be confirmed, however, by Hunter *et al.* (171). These authors also pointed out that dinitrophenol does not accelerate aging, though this ought to be the case, were the mitochondrial ATP the critical factor, as dinitrophenol is known to enhance the breakdown of ATP. On the other hand, from the data of Siekevitz & Potter (93), dinitrophenol actually does not promote the release of mitochondrial ATP. Siekevitz & Potter (93) also made the interesting finding that under conditions of inactive phosphorylation, as induced by aging mitochondria in the presence of fluoride, only ATP and an unknown adenine nucleotide are reduced in concentration within the mitochondria.

The precise mode of action of ATP on the mitochondrial structure is far from understood. In Raaflaub's (210) concept ATP acts as a chelating agent rather than a supplier of energy, protecting, like Versene, the mitochondrial structure against Ca^{++} . Alternatively, as suggested by Ernster & Löw (129, 142, 172, 173), the role of ATP may be to maintain a certain energy level within the structure, necessary for the functional integrity of the mitochondrial enzyme system. The finding of Chappell & Perry (221) that dinitrophenol inhibits the ATP-induced reversal of mitochondrial swelling would

seem to favor the latter alternative. On the other hand, this finding may indicate that the active principle responsible for the maintenance of structure is not ATP per se, but a compound which stands in equilibrium with ATP through a reaction that is inhibited by dinitrophenol. What this compound is, and why it is destroyed when mitochondria are exposed to aging, are important future problems. The interesting observation of Hunter *et al.* (171) that anaerobiosis protects mitochondria against aging might indicate that the compound in question can be destroyed by oxygen.

Several investigators drew attention to a possible similarity between mitochondrial and muscle contractions. Raaflaub (210) wrote in 1953:

... wir (sind) geneigt, die Ursache der nicht-osmotischen Schwellung (der Mitochondrien) im Vorhandensein kontraktile und dilatationsfähiger Strukturelemente zu suchen. Ein Vergleich mit dem trotz enormen Arbeitsaufwandes immer noch ungelösten Problem der Muskelkontraktion drängt sich auf, indem bei beiden Vorgängen ATP der im Zentrum des Interesses stehende Stoff ist.

Ernster (104, 142) pointed out that the presence of a contractile principle in the mitochondrial structure may play the role of a regulator of the extent to which generated high-energy bonds are exposed to hydrolysis, and may thus constitute a basis for metabolic control. Some time ago Perry & Chappell (225, 226) commenced a most valuable comparative study of the mitochondrial and myofibrillar ATPases, based on the previous observation (227, 228) that the Ca^{++} -activated ATPase of myosin, in analogy to the ATPase of resting mitochondria, can be stimulated with dinitrophenol. In these studies the interesting finding was made that the stimulating effect of dinitrophenol on myosin ATPase can be abolished by the addition of actin.

Witter & Cottone (229, 230) showed that lysolecithin is a potent inducer of mitochondrial swelling. They suggested that the aging effect studied by other authors might be due to the activation of a hypothetical mitochondrial lecithinase, inhibited by ATP or Mg^{++} . These assumptions not only lack experimental foundation, but they are also made improbable by the fact that succinic oxidase, which is known to be highly sensitive to lecithinase (231), is one of those few mitochondrial activities that is not affected by aging (172, 210).

Miscellaneous data.—Because of space limitation, no detailed review could be made of a number of valuable papers on topics relevant to the role played by mitochondria in various enzymic mechanisms. Some of these topics are: localization and mechanism of nucleotide transphosphorylations (232 to 234), mechanism of action of mitochondrial oxalacetic carboxylase (235), oxidation of tartrate in rat liver mitochondria (236, 237), mechanism of conversion of cysteinsulfinic acid into pyruvate and sulfite (238), conversion of sarcosine into serine and "active C_1 " (239), intracellular distribution of choline acetylase in brain (240), enzymic mechanisms of insect flight muscle mitochondria (241, 242).

PHYSIOLOGY AND PATHOLOGY

Variations in number and structure.—In the adult cell, the number of mitochondria seems to vary with the size of the cell. A rat liver cell contains an average of 830 mitochondria according to independent data of Lowe *et al.* (243) and Allard *et al.* (244). In eggs of two sea urchin species, mitochondrial counts of 14,000 and 33,000 were reported by Shaver (245). In the giant amoeba, *Chaos chaos*, Andresen (246) found 2 to 500,000 mitochondria per amoeba, with a straight-line relationship between individual amoeba volumes and mitochondrial counts. In a noteworthy study of the quantitative relations between liver mitochondrial metabolism and total body weight in mammals, Smith (247) concludes:

The evidence available appears to establish beyond reasonable doubt that, at least in mammalian liver, the mitochondrial amount bears essentially the same quantitative relation to total metabolism as to total body size, and it appears probable that the relative amounts of these elements in any given tissue will prove the controlling factor in determining the regression of oxygen utilization on total body size of the species.

Gustafson's (248) concept that embryonic differentiation is intimately connected with intracellular reorganization processes taking place at the mitochondrial level has been largely substantiated and extended (245, 249 to 251). Of special interest are the findings of Weber (252) that in the *Tubifex* embryo, ectodermal and endodermal mitochondria markedly differ with respect to structure, intracellular orientation, and accessibility to histochemical tests, and those of Boell & Weber (253, 254) that the cytochrome oxidase content per mitochondrion undergoes a substantial increase during the early development of *Xenopus*. The opposite of the latter process, as indicated by a decrease of the respiratory activity per unit mitochondrial protein, has recently been reported to occur in the liver of the aging rat (255).

Several attempts have been made to correlate experimentally evoked hormonal changes and other physiological disturbances with altered mitochondrial patterns. Lowe *et al.* (243) reported that cortisone treatment of rats results in a marked reduction of the number of liver mitochondria, accompanied by a swelling as revealed by phase contrast microscopy. In parallel studies (256), however, no decrease was found in the ability of the isolated mitochondria to oxidize succinate or to carry out oxidative phosphorylation. A reduced liver mitochondrial number in cortisone-treated rats was also reported by Allard *et al.* (244). Hartmann (257) investigated the fine structure of human and rat brain and the effect of cortisone on the latter. The mitochondria, which form transverse crests in the perikaryon area of the nerve cells, become vacuolized upon low doses of cortisone, and the number of crests becomes reduced; upon high cortisone doses, the mitochondria swell. No similar changes were observed in the glial elements.

A careful electron microscopic study of the effects of hypophysectomy on

rat adrenal cortex has been presented by Lever (44, 49). Following hypophysectomy there occurs a marked reduction of the number and osmophilia of the mitochondria together with a loss of the cytological definition. These changes could be restored by a temporary lowering of the Na/K ratio in the diet, or, in a more widespread manner, by ACTH treatment. Reid and associates (258 to 263) have undertaken a series of investigations of some quantitative aspects of the effects of hypophysectomy and subsequent growth hormone treatment upon rat liver mitochondria. The most clear-cut result as yet obtained is that growth hormone treatment of hypophysectomized rats causes a three-fold increase in the number of liver mitochondria.

Howe *et al.* (264) found that in the mammary gland of the lactating guinea pig there is an increased number of mitochondria, but their size is smaller, the total mitochondrial mass being unaltered. Weiss (265) reported an elegant electron microscopic study of duodenal cation transport in mice fed large amounts of sodium or potassium ions. The changes caused by anoxia or hyperoxia in the fine structure of lung alveolar mitochondria were studied by Schulz (266).

The structural changes of liver mitochondria, connected with fasting and refeeding, carbon tetrachloride poisoning ("cloudy swelling"), or regeneration following partial hepatectomy, have been intensely studied by Gansler, Rouiller & Bernhard (24, 50, 51). The results of these studies are of great cytological interest and were therefore reviewed in the section on *Cytology*.

Howatson & Ham (267) performed a thorough electron microscopic study of two liver tumors. In confirmation of earlier data (268) there was a reduction of the number of mitochondria, and also of the membranes of the endoplasmic reticulum. The mitochondria showed a normal structure with well-marked *cristae*.

The fine structure of mitochondria and the structural changes leading to the formation and evolution of the mitochondrial body or *Nebenkern* of the spermatid of insects have been studied by Tahmisian *et al.* (269, 270), de Robertis & Raffo (35), and Baker (271).

Inhibitors and modifiers of mitochondrial activities, biochemical anomalies.—Among the large number of inhibitor studies published during recent years only a few were of purely biochemical interest. One of these is Montgomery, Fairhurst & Webb's (272, 273) excellent work on the action of parapyruvate on α -ketoglutarate oxidation. In most instances the investigations concerned agents of physiological, pharmacological, or toxicological interest, with the hope of finding a clue to their physiological mode of action. In a few cases, well-defined, specific effects could be ascertained, while in others the observed changes belonged to the well-known "aging" symptoms of isolated mitochondria. These symptoms consist, *inter alia*, of a lowered phosphate uptake or phosphate/oxygen ratio, a reduced ability to carry out DPN-linked oxidations (restored by added DPN), an increased resting Mg^{++} -dependent ATPase activity, and an increased tendency to swelling. Such changes can be

evoked, as known, by a great variety of means, for example by detergents, and not surprisingly, then, also by agents which happen to have a certain physiological or pharmacological interest. Moreover, when only one of the symptoms is recognized, for example a reduced phosphate/oxygen ratio, an unspecific effect can easily be mistaken for a specific one.

But the main difficulty in evaluating the physiological implications of this type of result often lies not so much in the decision of whether or not the observed effect is specific in the biochemical sense (this as a rule is easily done in expert hands), but rather in the assessment of the question as to whether the *in vitro* finding has a relevance to the physiological mode of action of the agent studied. This problem arises regardless of whether or not the *in vitro* effect is specific, the only difference being that in the case of a specific effect, concerning one single enzyme, there is a greater probability that the physiological mode of action ultimately is related to the *in vitro* effect than in the cases of a general structural labilization. In the latter case it cannot even be taken for granted that the *in vivo* action primarily concerns the mitochondrial structure and not some other cellular element. Unfortunately, hormones appear more and more to belong to the latter category.

But again, even in the case of a specific effect, and a good correlation between *in vitro* and *in vivo* potency, a serious difficulty is how to explain that the *in vivo* effect can be limited to one single organ when the agent in question acts on an enzyme present in all tissues. Certain derivatives of barbituric acid, for example, are well-established specific inhibitors of the mitochondrial DPNH-cytochrome-*c* reductase (132), with a good correlation between inhibitory potency and pharmacological activity (130). Thus, the relatively strong sedative, Amytal, is a strong inhibitor of mitochondrial respiration, the weaker pentobarbital less strong, while the pharmacologically inactive 3-hydroxy-amytal has virtually no effect on the respiration of isolated mitochondria. It is also established (274) that isolated liver and brain mitochondria are equally sensitive to Amytal. Still, Amytal is a narcotic, primarily, and not a liver poison. Does this mean that brain function is more dependent on full respiratory capacity than is liver function, or does it mean that the pharmacological activity of barbiturates is not connected with their action on the mitochondrial DPNH-cytochrome-*c* reductase?

Wade & Jones (275) investigated the effect of progesterone on isolated rat liver mitochondria. The hormone uncoupled phosphorylation from respiration, with a slight inhibition of the latter, and with a stimulation of ATPase in both fresh and aged mitochondria. Testosterone, pregnanedione, and 17- α -hydroxyprogesterone were without effect. Dickens & Salmony (276, 277) studied the action of stilbestrol on oxidative phosphorylation in rat liver mitochondria. Again, an uncoupling of phosphorylation and a slightly inhibited respiration were the observed effects. Similarly to dinitrophenol (220), stilbestrol was found to protect isolated mitochondria against swelling caused by thyroid hormone. Working with mitochondria isolated from mouse

liver, brain, heart, and various tumors, Wight (278) found that dihydroxystilbestrol inhibits succinoxidase. The action on tumor mitochondria could be reversed with testosterone.

Kielley (279) and Emmelot (280) reported that the hepatic carcinogen, N-2-fluorenyldiacetamid, inhibits liver glutamic dehydrogenase. A comprehensive study of the effects of guanidine and decamethylenediguanidine (Synthalin) on isolated kidney mitochondria has been undertaken by Hollunger (281). These agents inhibit mitochondrial respiration when this is coupled to phosphorylation. The tranquilizer, chlorpromazine, had been reported by Abood (282) to cause a partial inhibition of phosphorylation in rat brain mitochondria; more recently Berger *et al.* (283) found no effect on phosphorylation in brain mitochondria, but a partial inhibition in liver. Abood & Romanek (284) brought about a partial uncoupling of oxidative phosphorylation by leading an electric current through a suspension of rat brain mitochondria; the effect could be prevented with glutathione, sodium sulfide, diethylthiocarbamate, or Versene. An uncoupling of oxidative phosphorylation in rat brain mitochondria was also reported to occur with salicylic acid and related compounds (285).

Zetterström & Ernster (286) found that bilirubin, but not biliverdin, uncouples oxidative phosphorylation in rat liver mitochondria. Evidence was presented indicating that the brain syndrome, kernicterus, in jaundiced newborn infants may be due to a toxic action of bilirubin (287). Aldridge & Cremer (288) studied the effects of certain tin compounds on rat brain and liver mitochondria. Diethyltin dichloride was found to specifically inhibit α -ketoacid oxidations, whereas triethyltin sulfate uncoupled oxidative phosphorylation, with a partial inhibition of DPN-linked oxidations. The mushroom poison, phalloidin, was reported by Hess (289) to inhibit the phosphorylation coupled to the oxidation of ferrocytochrome-*c*.

Emmelot and associates (290 to 293) carried out a laborious investigation of mitochondria prepared from various tumors with respect to biochemical organization and swelling properties. Several degenerative changes could be demonstrated, varying in degree according to the tumor used. Typical "aging" symptoms of the mitochondrial enzyme organization were also reported to occur in the mouse spleen after total body irradiation (294), in the syndrome of "cloudy swelling" of rat liver (295, 296), and in liver autolysis (297). Frei & Ryser (298) reported that vitamin B₁ deficiency leads to a structural labilization of rat liver mitochondria, while deficiency in vitamin B₂ is reflected in a reduction of the total liver mitochondrial protein. Liver mitochondria from cortisone-treated (256) or alloxan-diabetic rats (299) were reported to exhibit unaltered capacities of respiration and phosphorylation.

Thyroxine and metabolic control.—The chemical similarity between thyroxine and phosphorylation-uncoupling halo- and nitrophenols, and the recognition of the dependence of mitochondrial respiration on the availa-

bility of inorganic phosphate and phosphate acceptors (148, 300 to 303), stimulated a great deal of interest in studies of the action of thyroid hormone on mitochondrial activities. Early experiments appeared to support the view that thyroxine, applied *in vivo* and *in vitro*, uncouples mitochondrial oxidative phosphorylation by a dinitrophenol-like action [cf. (19, 21, 304, 305)]. By an extension of this view, Lardy (21, 306) suggested that thyroxine, and possibly also dinitrophenol, might act on the individual phosphorylations along the respiratory chain in a "stepwise" manner, strongest on the most tightly coupled, and weakest on the least tightly coupled phosphorylation. It was postulated that the physiological action of thyroxine on basal metabolic rate may be explained in terms of an increased expenditure of phosphate bond energy at the expense of thermodynamic efficiency. However, all efforts as yet to localize the thyroxine action to one individual phosphorylation have been unsuccessful (137, 307, 308).

Recent developments in this field have revealed some marked differences between the modes of action of thyroxine and dinitrophenol on oxidative phosphorylation. Thus, as shown by Tapley *et al.* (194, 309), thyroxine, in contrast to dinitrophenol, uncouples oxidative phosphorylation only at the mitochondrial level, while the dinitrophenol effect ensues in mitochondrial as well as in submitochondrial systems [cf. (206)]. A further difference is that thyroxine accelerates, whereas dinitrophenol delays the swelling of isolated liver mitochondria (194, 220, 309). The effects of thyroxine on both swelling and oxidative phosphorylation can be prevented by Mg^{++} , Mn^{++} and Versene (220, 310), and accentuated by Ca^{++} (311). All these facts indicate that thyroxine acts on mitochondrial oxidative phosphorylation through a labilization of the mitochondrial structure rather than by a specific, dinitrophenol-like mechanism. This view is further supported by the fact that the liver mitochondria of hyperthyrotic animals are swollen (312) and show a disorganized fine structure in the electron microscope (313), and also by the recent findings that, besides oxidative phosphorylation, thyroxine also affects a number of pyridine nucleotide-linked reactions (314). Of special interest is a short report by Phillips & Langdon (315) that thyroidectomy (or hypophysectomy) reduces, and subsequent thyroxine treatment restores the level of liver TPNH-cytochrome-*c* reductase. Moreover, as found by Tapley & Cooper (316), the susceptibility of isolated mitochondria to thyroxine-induced swelling varies from tissue to tissue according to the same pattern as does the uptake of I^{131} -thyroxine *in vivo*.

This state of affairs seems to necessitate a search for new lines of thinking in attempting to understand the cellular basis of thyroid action. These may have to take into account, besides the quantitative thermodynamic consequences of a less tightly coupled oxidative phosphorylation, the possible qualitative implications of an altered mitochondrial structure for cellular metabolism. After all, even the normal liver cell's mitochondria are "thyroxine-treated," and this state of thermodynamic imperfection is perhaps a re-

flection of the great crux in cellular energy economy that foodstuffs have to serve not only as fuel but also as building blocks of living material [cf. (1, 129)]. As pointed out by Hoch & Lipmann (308):

... for an orderly synthetic activity, balance is needed between hydrogenation and condensation reactions. Considering, for example, fatty acid synthesis, this would become impossible, if all substrate hydrogen were forced into conversion to phosphate-bond energy. No hydrogen, then, would remain available for the two hydrogenation reactions which have to follow every condensation step. It may thus be visualized that the hormone regulates the system in such a manner as to allow enough hydrogen to escape transformation to balance properly hydrogenation and condensation against each other. In this manner a loosening of a tight coupling would be beneficial and would indeed allow synthetic reaction to proceed more smoothly.

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GROWTH (HORMONAL REGULATION)^{1,2}

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This review is concerned with aspects of the hormonal regulation of growth in animals. It deals with recent work on the hypophysial growth hormone, on insulin and thyroxin, on testosterone, on limited but relevant aspects of the hormones of the adrenal cortex, and on certain interesting hormonal interrelationships. The discussion is further limited to the actions of the hormones that seem to be most directly related to growth or to protein metabolism, or to the function of those organs of assimilation, distribution, and excretion upon which the growth of an organism may be intimately dependent. The labyrinthine paths of carbohydrate and lipid metabolism have therefore not been followed; it would be impossible in the allotted space, and in any event we cannot lay claim to the insights required to select the work most relevant to the problems of growth. Some of the reviews cited in the following text deal effectively and in detail with this work. The review deals mainly with work appearing since 1953 and until June, 1957. It is too much to hope that we have not overlooked some work worthy of mention in the welter of the literature, and we ask to be forgiven for this. It may be thought that we have dealt brusquely with some matters, and dismissed certain fond notions too easily. For this exercise of auctorial opinion we ask no forgiveness but merely for an opportunity of discussion at pleasurable length in a more spacious room.

GROWTH HORMONE

The subject of the nature and actions of anterior pituitary growth hormone was reviewed by a number of authors in an international symposium published in 1955 (1), by Astwood (2) and more recently by Ketterer, Randle & Young (3). Detailed accounts of the chemistry of bovine growth hormone have been presented by Li (4) and by Hays & Steelman (2).

The most interesting development in this field has been the discovery that there is a degree of specificity among the growth hormones of different species of animals. This is indicated by the observations that normal and hypophysectomized guinea pigs fail to respond to an active bovine growth hormone preparation (5), that preparations from fish pituitary glands are not active in the rat [Wilhelmi (1)] although both fish and bovine growth hormones are active in intact and hypophysectomized fish [Pickford *et al.* (6 to 10)], and that both intact and hypophysectomized monkeys fail to respond to bovine growth hormone by the retention of nitrogen, by the

¹ The survey of literature pertaining to this review was completed in June, 1957.

² The following abbreviations are used in this chapter: COLA (cortisol acetate); DOCA (deoxycorticosterone acetate); STH (somatotropic or growth hormone).

changes in carbohydrate metabolism which are so easily elicited in the dog, the cat, or the rat, or by characteristic changes in the costochondral junction [Knobil *et al.* (1, 11 to 15)]. There is in addition the long series of reports on the disappointing effects of bovine and some pig growth hormone preparations in human subjects (1, 16 to 24). [A recent report, that of Prudden, Pearson & Soroff (25) concludes that bovine growth hormone is able to augment nitrogen retention in man during convalescence from severe burns, but the data as published do not support the claims of statistical significance.] It has now been shown that purified monkey pituitary growth hormone is active in bringing about nitrogen retention in young hypophysectomized monkeys (26) and that preparations of both monkey and human growth hormone, made by the glacial acetic acid extraction method of Raben & Westermeyer (27, 28) are highly effective in establishing marked positive balances in nitrogen, phosphorus, calcium, potassium, and sodium in the human (29). The subject, a 13-year old male weighing 73 lbs. with hypopituitarism secondary to craniopharyngioma, responded to as little as 10 mg. per day of the human growth hormone preparation and 40 mg. per day of the monkey growth hormone preparation, administered intramuscularly every 6 hr. There was an increase in urinary aldosterone excreted during the periods of treatment with both hormone preparations.

At least part of the physical and chemical basis for the differences between the bovine and the monkey and human growth hormones has been established by Li & Papkoff (30), who have described a method for preparing the pure hormones and have published data on their amino acid composition, molecular weights, isoelectric points, and nitrogen-terminal groups. The purified hormones from the three species all have the same order of biological activity in the tibia test in the hypophysectomized rat. Bovine growth hormone has a molecular weight of 46,000, contains 396 amino acid residues, has two nitrogen-terminal groups, alanine and phenylalanine, and one carbon-terminal group, phenylalanine, and is isoelectric at pH 6.85. Monkey and human growth hormones have molecular weights of about 26,000, contain 241 to 245 amino acid residues, have only one nitrogen-terminal group, phenylalanine, and much more acidic isoelectric points, at or near pH 5.5. There are, further, small differences in detail (as for instance in the number of cystine residues per molecule—four and two, respectively) between monkey and human growth hormone. Similar marked differences from bovine growth hormone have been noted by Wilhelmi for the growth hormones from two species of fish and from pig pituitaries (1). As judged by bioassay in comparison with bovine growth hormone in rats, the adult human pituitary contains considerable quantities of this material (31, 32). The yields of growth hormone from monkey pituitaries are also unusually high (28, 30).

The existence of species specificity to some degree among the growth hormones raises many interesting questions. It is known at present that the growth hormones of ox, pig, horse, sheep, monkey, and man are all effective

in the common test animal, the rat, but that the growth hormones of at least two species of fish are not. Bovine growth hormone, however, is active in the fish and frog as well as in the cat, dog, rabbit and mouse. No nonprimate mammalian growth hormone has been unequivocally active in man. The question to be resolved is whether in the progress from fish to amphibian to mammal to primate, structural changes in the growth hormones have evolved such that the hormone from a higher species is effective in a lower one, but not the other way round. This would mean that the "active center" of the hormone has become progressively more complex in going from fish to primate. Another possibility arising from the broad species range of action is that there is a common structure conferring activity, and that the specificity is conferred by the remainder of the molecule. Whether the hormone is effective or not may then depend upon whether the difference in the molecule is great enough to interfere, or whether the animal receiving the hormone can modify it. This somewhat more hopeful hypothesis of an "active core" common to the various growth hormones arises from the observations of Li and his colleagues (4, 33) that bovine growth hormone may be partially digested (to the extent of 24 to 30 per cent) with chymotrypsin or trypsin without significant loss of biological activity. The digest no longer contains any material identifiable by physical or chemical means as the original hormone. The resolution of this mixture and the isolation and definition of the "active core" may be awaited with great interest.

Bioassay.—The problems of bioassay of growth hormone have been studied by Russell (1) and, with special reference to the tibia test, by Geschwind & Li (1). Lostroh & Li (34) have found the tibia of the hypophysectomized mouse to be a reliable and sensitive test object. Amounts of growth hormone detectable by a modified rat tibia test have been reported in the plasma of calves and young pigs, in retroplacental human blood, and in the plasma in several cases of gigantism and acromegaly, but not in normal human plasma (35, 36). Gemzell *et al.* (37) assayed growth hormone in the plasma of normal rats injected with 1 mg. per 100 gm. of bovine hormone, finding an initial concentration of about 140 microgram per ml. and a half-life of about 40 min. After the injection of moderate doses of I^{131} -labelled growth hormone into rats, the radioiodine is localized in most of the internal organs, particularly in the pancreatic islets and in the adrenals, but not specifically in the tibial epiphysis as compared to the rest of the bone [Sonenberg *et al.* (38)].

Nitrogen metabolism.—A number of contributions have been made to the problems of the effects of growth hormone on general growth and nitrogen metabolism. Several papers extend the view, first clearly demonstrated by Gordan *et al.* (39, 40), that the effect of growth hormone is dependent upon an adequate supply of proteins and amino acids and of certain vitamins. Growth hormone was observed to have no effect on the rate of weight loss in rats during protein depletion, but it exerted its customary effects during repletion on a diet adequate in protein (41). In the nephrectomized rat given

amino acid hydrolysate intravenously, the administration of growth hormone 1 to 2 hr. beforehand results in a lower rate of accumulation of urea in the blood [Russell (1, 42)]. This acute effect is related to the dose of the hormone, and the minimum effective dose is of the same order as that required to elicit nitrogen retention in the intact rat. No effect is seen in the absence of amino acid hydrolysate, nor is the effect influenced by the simultaneous administration of ACTH. In eviscerated rats given amino acid hydrolysate, growth hormone increased the apparent volume of distribution of the administered amino acids although it had no effect on the rate of rise of blood or plasma amino nitrogen when no amino acids were given (43). The amino acid mixture necessary for the support of these effects need not be of exogenous origin, if one may judge by the experiments of Volk & Lazarus, who observed a marked effect of growth hormone on nitrogen retention in the fasting rat whose nitrogen catabolism had been greatly increased by phloridzin but no effect in the normal fasted rat or in the phloridzinized adrenalectomized rat (44). In harmony with this are the observations that the fasting nitrogen excretion is greater than normal in the first 48 hr. after hypophysectomy in the rat and that this extra nitrogen output may be suppressed by growth hormone (45). One of the few reports of a positive effect of bovine growth hormone in humans is that of Carballeira *et al.* (46). In patients given an intravenous infusion of amino acids, bovine growth hormone resulted in a lowered plasma and urine amino nitrogen, but the hormone had no effect if the infusion of amino acids were omitted. In these experiments the hormone, in doses of 1, 2, or 3 mg./kg., was also injected intravenously. Growth hormone is found to be without effect in animals deficient in pyridoxine, riboflavin, or pantothenic acid, but some effect, possibly correlated with food intake, was seen in the thiamine-deficient rat (47, 48, 49).

There have been two brief reports that treatment of rats with growth hormone results in diminished urea synthesis by liver slices (50, 51). It has also been reported that the excretion of conjugated benzoic and phenylacetic acids was unchanged in dogs treated with growth hormone and retaining nitrogen (52).

The effects of hypophysectomy and of growth hormone on the uptake and distribution of labelled amino acids have been reported by a number of workers. The uptake of S^{35} -labelled cystine into tissue proteins was diminished in hypophysectomized rats and increased by treatment with growth hormone (53). Normal rats, treated with growth hormone for a week, took up a greater proportion of cystine- S^{35} into the nuclear and mitochondrial fractions of the liver, in contrast to the regenerating liver in which there was diminished uptake by the nuclei and normal uptake by the mitochondria (54). Bartlett *et al.* observed an increased uptake of N^{15} -glycine into the plasma protein of dogs treated with growth hormone (55). In a series of papers (56 to 58), this group also reports on the effects of growth hormone on the excretion of isotope from N^{15} -glycine, using mainly the experimental

design and method of analysis of Sprinson & Rittenberg (59). They find that in dogs in positive nitrogen balance as a result of treatment with growth hormone the proportion of glycine- N^{15} excreted as urea was diminished, an effect which is interpreted to mean an increase in protein synthesis. There did not seem to be a significant effect in animals given growth hormone during a 5-day fast. In these latter experiments (58) the mode of presentation of the data (largely derived) makes them difficult to evaluate. Treatment with growth hormone may have induced a slightly greater uptake of N^{15} -alanine into plasma fibrinogen and the proteins of several tissues of hypophysectomized rats (60). The data do not permit one to evaluate the possibility that the isotope may have been somewhat less diluted in the treated animals. Prudden *et al.* (61) have reported experiments on isolated perfused rabbit liver, using C^{14} -glycine included in an amino acid mixture. From their combined chemical and isotopic data they concluded that growth hormone is a "powerful anabolic agent," but the data are not presented sufficiently clearly or in enough detail to be at all convincing.

The distribution and turnover of plasma albumin in the hypophysectomized or growth hormone treated rat has been studied, using S^{35} -labelled albumin, by Ulrich, Tarver & Li (62). In these experiments the shape of the specific activity curves indicated that the turnover of plasma albumin was not simple, and that there must have been return of isotope from other compartments. In the treatment of the data certain assumptions were expressed or implicit. The first assumption, which may be approximately true, was that the animals were in a steady state during the period of observation. Second, the plasma albumin is assumed to equilibrate with another substantial pool of albumin which is not circulating. Thirdly, it is assumed that the circulating and noncirculating pools of albumin have identical relative turnover rates, i.e., that the rate of entry of newly synthesized unlabelled albumin into each of these pools is proportional to the size of the pool, and that the rates of catabolism are similarly related. This last assumption, which may be unlikely but is not impossible, is not supported by any independent evidence. With these assumptions, it is calculated that the circulating pool in all cases is about one-third of the "total exchangeable albumin." The total exchangeable albumin was not affected by hypophysectomy but was increased after two weeks of treatment with growth hormone. The total replacement rate was about half normal in hypophysectomized animals and was restored to normal or above by growth hormone.

Composition of tissue.—A detailed analysis of the effects of growth hormone on the weights of various organs and of individual muscles of the rat has been presented by Greenbaum & Young (63). All organs but the heart and soleus muscle increased in size with chronic treatment. The internal organs were relatively little affected, and the response of individual muscles varied very widely. A study of one of the more responsive muscles—quadriceps—revealed no changes in the proportions of sarcoplasm, myofibril, or collagen and elastin fractions, but the ATP ase activity of the myofibrils

per gram of tissue was less than normal (64). In this connection it has also been observed in growth hormone-treated rats that the functional capacity of quadriceps is the same as or slightly less than normal in spite of the increase in size (65). In rats hypophysectomized at weaning the composition of the main protein components of thigh muscle remained normal, but after treatment with growth hormone, which increased the mass of the thigh muscle about in proportion to the increase in body weight, there was a relative increase in myosin [Scow (66)].

It has now been shown, in confirmation of earlier work with crude pituitary extracts, that chronic treatment of normal animals with purified growth hormone induces increased deposition of protein together with water and a fall in the proportion of body fat (67, 68). In dogs given diabetogenic doses of growth hormone, Campbell *et al.* observed not only nitrogen retention but also considerable increases in the total amounts of the plasma proteins (69). The often-observed fall in plasma amino nitrogen induced by growth hormone has been studied in relation to other hormonal factors by Milman *et al.* (70), by Luck and coworkers (71) and by Russell, who has discussed this in relation to other aspects of the action of growth hormone (72).

The effects of hypophysectomy and of growth hormone on nucleic acids have received a growing amount of attention. DiStefano *et al.* (73, 74), using both histochemical and chemical methods, observed that the concentration of RNA is reduced in both nuclei and cytoplasm of liver cells of hypophysectomized rats, and that the RNA is restored to normal by treatment with growth hormone over two to six days, the cytoplasmic RNA being the more slowly restored. The amount of DNA per nucleus appeared to be unchanged in the hypophysectomized animal. The latter is disputed by Lison & Valeri (75) who claim a small but significant decrease in the DNA of the most numerous type (II or B) of nucleus. But Bass *et al.* have found that the DNA of liver nuclei is increased after hypophysectomy, the effect being associated with an increase in the proportion of diploid and octaploid nuclei and a decrease in tetraploid nuclei (76). The point is somewhat clarified by the observation that in young rats (75 gm.) a shift from predominantly diploid to predominantly tetraploid liver nuclei may occur within a short period (8 days), but does not occur if the animal is hypophysectomized at this time (77). This change occurs normally, however, if growth hormone is administered. A more detailed study of RNA and DNA in the liver cell components has been made by Reid (1, 78). The decrease in RNA after hypophysectomy occurs both in the cell as a whole and in the mitochondria. Growth hormone restores the mitochondrial RNA and also brings about a shift of RNA from the microsomes to the supernatant fraction. There were no significant changes in DNA. None of these changes were seen in kidney tissue. An attempt to study the dynamics of the changes in liver with leucine-C¹⁴ and orotic acid-C¹⁴ failed to reveal any clear-cut effects of growth hormone (79). In plateaued adult female rats growth hormone brought about

an increase in RNA per gm. of nitrogen in muscle tissue [Gray (80)]. There were no changes in muscle DNA, and no change in either constituent of the liver. The 6-hr. incorporation of P^{32} into nucleo-proteins and into phospholipides was increased in parallel in the livers of rats treated with growth hormone for 10 days (81).

A summary of the effects of hypophysectomy and of growth hormone on tissue enzymes has been presented by Gaebler (1). Very little of significance seems to have been reported since (51, 60, 82 to 89). It may be pointed out that all of the effects observed occur only after some days of treatment, and that they may therefore be taken to be effects rather than causes of the altered metabolism induced by the hormone.

A number of workers agree in the observation that after hypophysectomy many enzymes and secretions of the alimentary canal are much diminished, but there is less general agreement about which hormone or hormones may be required to restore these to normal (90 to 95). This subject has been reviewed recently by Baker (1, 96).

Bone and cartilage.—The role of hormones in the growth and maturation of bone has been outlined in a fine review by Simpson *et al.* (97). This same group has reported on the effects of very early hypophysectomy on the skeleton, and on the effects of treatment with growth hormone (98 to 101). The skeleton grew to some degree but the soft tissues to a somewhat greater extent in the absence of the pituitary; the brain tended to outgrow the calvarium. Growth hormone increased the size of the skeleton, which, however, remained immature. In the absence of the hypophysis there was no regeneration after removal of a portion of the calvarium, whereas in normal animals or in hypophysectomized animals treated with growth hormone there was extensive regeneration (102). The effects of long-continued administration of growth hormone on the skeleton of intact rats have been reported in detail by Asling *et al.* (1). The effects were strikingly similar to those seen in acromegaly. The bone and joint changes in this disorder have been nicely described by Kellgren, Ball & Tutton (103).

The uptake of S^{35} -sulfate into cartilage is depressed after hypophysectomy and increased by growth hormone (104 to 106). These observations have been confirmed for cartilage *in vitro*, with the very interesting addition that growth hormone itself has no direct effect *in vitro*, but that the uptake of S^{35} -sulfate is augmented by incubation with serum from normal animals or from hypophysectomized animals treated with growth hormone (106a). The uptake of Ca^{45} into tibia is decreased after hypophysectomy and increased after treatment with growth hormone, with marked concentration of the isotope in the region of the epiphysis (107, 108). There was no effect in normal animals given growth hormone. Daily injections of growth hormone prevented the fall in tibial glycogen which occurred after hypophysectomy (109).

Hematopoiesis.—Additional observations have been made on the effects of growth hormone on the blood-forming organs. It is generally agreed that

growth hormone alone does not repair the anemia of hypophysectomized animals, although some effect may be inferred from the observation of increased plasma volume without change in hematocrit or of an increase in circulating hemoglobin in proportion to body size (110 to 113). This is consistent with the report that growth hormone leads to repair of the hypoplastic bone marrow and to an increased reticulocytosis (112 to 114).

Renal function.—The effects of hypophysectomy and of growth hormone on renal function have been reviewed by White (1). It is generally observed that glomerular filtration rate, renal plasma flow, and clearances are much depressed in hypophysectomized animals, and that chronic treatment with growth hormone tends to correct these deficiencies (115 to 119). An exaggeration of these effects, which could be correlated with increase in the size of the glomeruli and proximal convoluted tubules, has been observed in patients with acromegaly (120, 121). The concentration of *p*-aminohippurate by rat kidney slices was much reduced after hypophysectomy and restored to normal by treatment of the rats with growth hormone (122). Compensatory renal hypertrophy after removal of one kidney was not seen in hypophysectomized rats and dogs, but it did occur to a considerable degree during treatment with crude anterior pituitary extracts (123).

Neoplastic and embryonic growth.—The role of hormones in experimental tumorigenesis has been reviewed by Gardner (124) and by Kirschbaum (125). The part played by growth hormone in this process is as yet by no means clear. It has been reported that in some instances hypophysectomy diminishes the incidence or the growth of tumors induced by a variety of carcinogens or by inoculation of transplants (126 to 133), but it has also been reported that the induction of cancer by benzpyrene injected subcutaneously occurs as readily in hypophysectomized as in normal animals (134). There seems to be general agreement that growth hormone has little effect on the incidence, size, or distribution of tumors induced by carcinogens or transplants in intact rats or mice (126, 135 to 138), except for the report of Wood *et al.*, who find that injection of the hormone beginning 3 days prior to intravenous inoculation of a sarcoma leads to an increase in number and size of tumors in the lung (139). These same authors observed no effect of the hormone if injections were begun after the intravenous or subcutaneous inoculation of the sarcoma. There have, however, been some claims that treatment of hypophysectomized animals with growth hormone does increase the incidence of induced tumors (126, 130, 133). The significance of many of the observations made on operated animals is doubtful because the part played by inanition has not always been properly evaluated, because of the poor survival of the hypophysectomized animals, and because of the small numbers of animals observed in many of these experiments. In intact male rats injected with growth hormone for 14 months, a high incidence of pheochromocytomas was observed, but no higher incidence of other kinds of tumor than in control animals of the same ages, which exhibited no pheochromocytomas (140).

There is a small but interesting group of reports on the effects of hypophysectomy and growth hormone on foetal growth. Knobil & Caton observed that in rats hypophysectomized on the 12th day of pregnancy the young at term were smaller than those of sham-operated pair-fed controls (141). An increase in the size of the offspring has been observed in normal young female rats given purified growth hormone throughout pregnancy (142 to 144). Small amounts of growth hormone injected into incubated fertile eggs at day 13 resulted in the production of significantly heavier chicks at hatching (145). Application of growth hormone to explants of bone from chick embryos in tissue culture has no effect [Fell (1)].

Miscellaneous.—Emerson & Emerson have attempted to find an explanation for the falling off in rate of gain of weight of animals during continued treatment with growth hormone (146 to 148). They find that "plateauing" occurred in rats given extracts of rat pituitaries as well as crude or purified bovine growth hormone, and that it also occurred in adrenalectomized rats maintained on 11-oxy and 11-desoxy corticosteroids in various proportions.

Studies of the factors influencing hypertrophy of the heart in rats with an aortic constriction have been reported by Beznák (149 to 153). Enlargement of the heart does not occur in hypophysectomized rats, although the loss in weight of the heart that normally follows the operation does not occur if the aorta is constricted. Treatment of the animals with lyophilized anterior pituitary powder or with partially purified bovine growth hormone restored the capacity of the heart to hypertrophy. However, in later work, two highly purified bovine growth hormone preparations failed to exert this effect, although a somewhat less pure growth hormone from pig pituitaries was highly active. The effect was not produced by preparations of ACTH or by adrenocortical hormones.

Huggins, Parsons & Jensen observe that the preputial glands of the rat atrophy markedly after hypophysectomy, only slightly after gonadectomy and not at all after adrenalectomy (154). The atrophied glands are restored by treatment with either growth hormone (which did not itself affect the weight of the seminal vesicles or prostate) or androgens, the two hormones acting synergistically. Lostroh & Li report that purified bovine growth hormone seems to maintain the normal histological appearance of the secretory epithelium of the accessory sex glands in hypophysectomized rats (155). These authors also note that growth hormone has a small but definite effect on the weight of the adrenals, and that it strikingly augments the effect of ACTH on adrenal weight when it is given simultaneously with the trophic hormone. The effects are far more than additive. Similar effects of growth hormone, both alone and with ACTH, have been reported by Cater & Stack-Dunne (156). These authors also report that there is a burst of mitotic activity in the adrenal cortex a few hours after a single injection of growth hormone, and that with repeated injections at 8-hr. intervals the mitotic activity at first declines and then rises again to much higher levels in 24 hr. (157). Kalter, Stuart & Tepperman found that either growth hormone or

testosterone brought about a more rapid proliferation of influenza virus in mice (158). Hayashida & Li have observed that treatment of young Long-Evans female rats with growth hormone greatly augmented the titre of antibodies formed against Fraction IV A from *Pasturella pestis* (159).

A number of observations relating growth hormone to body water and electrolytes are worthy of note. Bennett and coworkers have reported that growth hormone induces retention of sodium, potassium, and chloride in both normal and adrenalectomized rats (160, 161). In hypophysectomized rats growth hormone increases the plasma and extracellular fluid volume and raises the low muscle potassium to normal (162). In a study of 18 patients with acromegaly, Ikkos, Luft & Sjögren have found very large increases in extracellular and total body water and in exchangeable sodium (163).

THYROID, PANCREAS, AND ADRENALS

Thyroid.—The relationship of the thyroid hormone to growth has two main themes. First, although the hormone itself will induce a minor degree of growth, for a full growth response both thyroxine and growth hormone are required. The manifestations of the two hormones differ notably but are complementary: thyroxine accelerates maturation without much influence on growth, growth hormone accelerates growth without effect on maturation. Second, for the normal production of growth hormone by the pituitary, normal thyroid function is essential. These themes are illustrated in the review by Simpson, Asling & Evans, cited above (97), and have been confirmed and extended by more recent work. Asling *et al.* (164) have reported that in rats hypophysectomized at 28 days the daily injection of 2 to 3.5 micrograms of thyroxine for a year brings about only small gains in weight and length, most of which take place early in the course of treatment, but a considerable degree of skeletal maturation. The daily injection of growth hormone for a two-month period at the end of the year brought about rapid gains of weight of the same order in both untreated and thyroxine-treated rats; previously untreated rats given both thyroxine and growth hormone during this time appeared to be more sensitive to the augmenting effects of thyroxine. Eartly & LeBlond (165) have presented a detailed study distinguishing between those effects of thyroxine which are direct (metabolic rate; changes in the skin), effects which require the hypophysis (body and skeletal growth, sexual development and growth of the adrenal gland) and effects which are incomplete in the absence of the hypophysis (growth and development of the heart and abdominal organs). As a further example of these relationships, it has been found that mitotic activity in the intestinal mucosa of rats is depressed after both thyroidectomy and hypophysectomy, restored to normal by thyroxine only in thyroidectomized rats, but restored by growth hormone in either group of operated animals (166). Young thyroidectomized rats eating *ad libitum* retained only about one-tenth of the amount of nitrogen retained by normal controls; even when force-fed at the

control level of intake they still retained much less nitrogen [Scow (167)]. Rupp, Paschkis & Cantarow (168) have confirmed by studies of nitrogen balance the failure of thyroxin to induce growth and nitrogen retention in hypophysectomized animals, although thyroxin was quite effective in thyroidectomized animals. In very young hypophysectomized rats thyroxin induced a very limited amount of growth and nitrogen retention (one-third of which was in the pelt) (169). Crispell *et al.*, measuring excretion of isotopic N after administration of N^{15} -glycine, concluded that protein synthesis was depressed in myxedematous patients and only rather slowly restored towards normal during therapy with triiodothyronine (170). Fell & Mellanby have found that both thyroxin and triiodothyronine, present in the medium in physiological concentrations, stimulate maturation of cartilage in embryonic chick limb bones in tissue culture (171, 172). Stimulation depends on the age of the bone, and differs in different bones. As the cultures aged, toxic effects—retardation of growth and cellular degeneration—were seen.

Pancreas.—It is well known that insulin is required for nitrogen balance or net nitrogen retention [first definitively demonstrated by Chaikoff & Forker (173)], and to prevent the rise in plasma amino nitrogen which otherwise occurs in hepatectomized animals [most recently demonstrated in dogs by Bollman *et al.* (174)]. The mechanism of this effect on nitrogen retention is not known, nor has any direct relation of insulin to growth yet been established. Munro, in an excellent review of the problem (175), considers that insulin is essential for the nitrogen-sparing action of carbohydrate, and furnishes evidence that this effect is exerted mainly in the peripheral tissues. Geiger & Pinsky (176) have reported that fructose and possibly glucose can spare nitrogen when fed together with protein in the alloxan-diabetic rat, but Ingle *et al.* (177) have found that fructose has no effect on the rise in plasma amino nitrogen which occurs in the eviscerated rat. Ingle *et al.* have shown that an actual fall in plasma amino nitrogen can be observed when large doses of insulin are given along with glucose to eviscerated rats (178). On the other hand muscle work, which also greatly increases glucose utilization, has no effect at all on the rise in plasma amino nitrogen in the eviscerated rat (179).

Forker & Chaikoff have reported that the turnover rate of S^{35} -methionine-labelled plasma protein in uncontrolled diabetic dogs does not differ from that in controlled animals (180). In functionally eviscerated, nephrectomized, diabetic dogs given S^{35} -labelled methionine, the uptake of isotope into muscle protein was only a fraction of that in control animals, and was restored to normal by prior treatment of the diabetic dogs with insulin for three days (181). The difference in these results suggest that the effect of insulin was on the synthesis of protein rather than its catabolism. Sinex, MacMullen & Hastings observed that insulin increased the uptake of C^{14} -alanine into the proteins of rat diaphragm *in vitro* in the absence of substrate, but, surprisingly, that the addition of glucose or pyruvate abolished the effect (182). Krahl, using C^{14} -glycine, has confirmed this observation on

the effects of insulin alone on the isolated diaphragm. He found, however, that the uptake of isotope into both liver and diaphragm was depressed in tissues from fasting normal or diabetic animals, and that the addition of glucose alone restored the uptake to normal. There was no significant effect of insulin on the liver in any case (183). Perhaps a more useful tool for the study of these effects may be the perfused rat liver, with which Miller and his colleagues have obtained convincing evidence of the sparing effect of carbohydrate on protein and amino acid catabolism (184).

Evidence that the nitrogen-retaining effect of growth hormone is dependent upon insulin, and may indeed depend upon an increased supply of insulin, has been presented by Milman, DeMoor & Lukens (70), Lukens & McCann (1), and Gaebler *et al.* (185). These workers agree that in depancreatized cats and dogs growth hormone does not cause nitrogen retention unless insulin is also supplied, and that the amount of insulin required is distinctly greater than that needed only to establish nitrogen balance. Consonant with these findings is other evidence that growth hormone may elicit an increased secretion of insulin in cats and dogs [Randle; Bennett (1)].

Salter & Best have reported that hypophysectomized rats given large daily doses of protamine zinc insulin together with a high carbohydrate diet exhibited large increases in food intake, some gain in weight, a positive nitrogen balance and moderate gains in body protein, very much greater increases in body fat, and increases in width of the tibial epiphyses (186, 187). When the effects of insulin and growth hormone were compared under conditions in which the gains in body weight were the same, it was noted that growth hormone induced much greater increases in body protein, a smaller accumulation of fat, and much greater increases in the width of the tibial epiphyses on a much smaller food intake. Similar observations have been reported by Salgado *et al.* (188). The latter noted also, however, that although the tibial epiphyses of the rats given insulin were somewhat wider than those of control hypophysectomized animals, but very little alteration in the atrophic state of the epiphyses was evident histologically; and they concluded that insulin did not exhibit a specific growth promoting effect comparable to that of the pituitary growth hormone. No one has as yet furnished the necessary controls for these observations by determining the effects on hypophysectomized rats of enforced food intake in the amounts and for the time experienced by the animals in these experiments. Salter & Best have suggested that, since insulin can bring about nitrogen retention in the hypophysectomized animal, it may in fact be the primary growth-promoting hormone, and this view has been taken up and elaborated by Ketterer, Randle & Young (3). Lukens & McCann (1), who have observed that in the Houssay animal the effects of insulin on nitrogen retention appear to be related to the amounts of glucose also retained, have offered an alternative interpretation emphasizing the complementary character of the effects of insulin and growth hormone on nitrogen metabolism.

The possibility that growth hormone excites the secretion of glucagon,

which might in turn account for some of the effects of the pituitary hormone on growth and metabolism, was first raised by Bornstein, Reid & Young, (189) who observed that the injection of growth hormone is followed by the appearance in portal vein blood of a substance causing a transient hyperglycemia in the diabetic, adrenalectomized, hypophysectomized rat. Foa & coworkers confirmed this observation in a series of cross-circulation experiments in dogs (190). Sirek, Sirek & Best have recently shown that the activity of the hyperglycemic substance elicited by growth hormone is blocked by dihydroergotamine (whereas that of glucagon is not), and further, that the hyperglycemic substance still appears after injection of growth hormone in the absence of the pancreas (191). In a recent note Sirek has presented evidence suggesting that the hyperglycemic agent could be 5-hydroxytryptamine (192). An effect of glucagon on the tibial epiphysis similar to that produced by growth hormone was claimed by Elrick (193), but this has been firmly denied by Geschwind & Staub (194) and by Mitchell, Rice & Girerd (195).

Adrenal cortex.—The catabolic effects of the adrenocortical hormones in excess are well recognized. There has, however, been some confusion about the part played by the adrenal cortex in normal growth or in the responses to growth hormone. Maassen (196) has found that young adrenalectomized rats maintained on small doses of desoxycorticosterone acetate (DOCA) gain in weight and in tail length if anything slightly better than do pair-fed intact animals given the same amount of DOCA. Cohn *et al.* have compared the composition of changes in body weight of adrenalectomized and normal rats eating *ad libitum* or force-fed equal amounts of food. The adrenalectomized animals were given salt solution to drink. The adrenalectomized rats eating *ad libitum* took less food, gained less nitrogen and lost more fat than the controls. In the force-fed groups, however, the gains in body weight, nitrogen, and fat were almost identical (197). Buffett & Wyman (198) compared the width of the tibial epiphysis, at a variety of ages, in normal and salt-maintained adrenalectomized rats. At each age the epiphyses were distinctly wider in the adrenalectomized rats, despite the fact that they ate somewhat less and grew more slowly. Stein *et al.* have found that the nitrogen retention induced by growth hormone in adrenalectomized rats maintained on saline or with adrenal cortex extract was the same as in normal animals (161). It is evident that if electrolyte balance and nutritional status of the animal are maintained, the adrenal cortex is not required for normal growth. The anabolic effects of small or moderate amounts of adrenocortical hormones in adrenalectomized animals are very likely wholly explicable as indirect effects related to hemodynamic state, general health, and nutrition.

As a result of a complex series of experiments on adrenalectomized rats bearing a granuloma pouch and treated with saline or with a range of doses of desoxycorticosterone acetate (DOCA), cortisol acetate (COLA) and growth hormone (STH) alone and in varying combinations, Selye has concluded that the responsiveness to growth hormone depends upon mineralocorticoids

and sodium chloride, and, somewhat surprisingly, "that the growth-promoting effect of STH cannot manifest itself in the presence of normal amounts of glucocorticoids such as are physiologically secreted by the adrenal cortex" (199, 200). In fact, if the groups receiving growth hormone are compared to their appropriate controls, when these can be found, it is seen that the gains in body weight are about the same in normal animals, in salt-maintained adrenalectomized animals and in adrenalectomized animals given DOCA in a wide range of doses. In adrenalectomized rats bearing a granuloma pouch COLA alone was not sufficient for complete survival or maintenance, no doubt because of the severe load on electrolyte and water metabolism imposed by the pouch. Where survival was possible in such animals, growth hormone reduced the loss in body weight. If then DOCA were given in addition to COLA, thus allowing full survival and some growth, the increase in body weight induced by growth hormone was of the same order as the "growth hormone effect" observed in the absence of DOCA. In these cases, the effect of growth hormone was less than in the comparable series without COLA, a reflection of the well-known mutual antagonism of the 11-oxycorticoids and growth hormone. No special effect of sodium chloride or of DOCA on the response to growth hormone can be seen in these experiments other than that to be expected from the improvement in hemodynamic state and nutritional status. These points are made clear in the following table of data

TABLE I
EFFECTS OF GROWTH HORMONE AND OF CORTICAL STEROIDS ON BODY
WEIGHT IN RATS BEARING A GRANULOMA POUCH*
[data from Selye (199)]
PER CENT CHANGE IN BODY WEIGHT

Treatment†	Un-operated	Adrenalectomized						
		NaCl	DOCA		COLA		DOCA .1 mg. COLA .4 mg.	DOCA 1 mg. COLA .25 mg.
			.1 mg.	1 mg.	.25 mg.	.4 mg.		
No growth hormone	+10	- 4 (3)‡	+ 4	+24	- 3 (2)	-23	+ 6	+14
Growth hormone 2 mg./day	+25	+14 (1)	+27	+41 (2)	-16 (7)	-14	+13	+23 (1)
Growth hormone effect	+15	+18	+23	+17		+ 9	+ 7	+ 9

* Ten rats per group, 12 days after induction of the granuloma pouch. There were no significant effects of growth hormone on the volume of exudate in the pouch in these experiments.

† Sodium chloride was provided as saline drinking fluid. The steroids were injected daily in the amounts given. DOCA: desoxycorticosterone acetate. COLA: cortisol acetate.

‡ Number died.

selected from (199). Additional evidence that DOCA does not enhance the action of growth hormone has been provided by Maassen (201). He observed that in young, pair-fed intact rats DOCA (0.5 mg./day) caused a small but significant reduction of the weight gain, and that in hypophysectomized rats DOCA (0.25 mg./day) had no effect on the weight gained in response to growth hormone.

SEX HORMONES

In a recent comprehensive book on the androgens, Dorfman & Shipley recount the work done until about 1953 on the relation of these hormones to growth and nitrogen metabolism (202). In recent years, work in this field has followed two main lines: the definition in detail of the pattern of growth induced by the androgens, and the study of the possibility of finding one or more steroids related to the androgens which have good protein anabolic effects but relatively weak or no androgenic activity.

The impression to be gained from past work, done mainly on the rat, is that the protein anabolic effect of the androgens is fairly general in character. This is illustrated by a recent report of Kochakian *et al.* that the changes in the major internal organs (except for kidney) and in 29 different skeletal muscles in the castrate rat were all in proportion to the changes in body weight (203). That this may not be true of all species is indicated by the work of Scow & Roe (204) and of Kochakian *et al.* (205, 206) on the guinea pig. In this species, the effects of gonadectomy or of androgens, apart from those intimately related to the sexual apparatus, are seen almost exclusively in the muscles of the head and neck. In the guinea pig, unlike the rat, the effects of castration on specific parts of the musculature (e.g., the temporal and masseter muscles) are quite marked, and it is thus easy to discern the relatively specific effect of androgens in restoring these muscles to or above normal size. Kochakian and coworkers have also observed that during a prolonged fast all of the muscles of the castrate guinea pig lose weight about in proportion to the loss of body weight, but that if testosterone in large doses (10 mg./day) is given to the fasting animals, the muscles of the head and neck, and to some extent those of the shoulder and upper back, do not lose weight to the same degree that the other muscles do (207). On the basis of these and related observations on the absence of effect of androgens on the thigh muscles of both rats and guinea pigs, Scow & Hagan have suggested that testosterone exerts its effects mainly on those muscles which are important in the sexual activity of the male of a particular species (208).

Korner & Young have presented detailed studies of the effects of the weak androgen, methylandrostenediol, on the organs and muscles and on body composition of normal male and female rats on constant food intake (209, 210). In both sexes, the weights of kidney, liver, and spleen were increased the most; next, the size of the acromiotrapezius muscle; small increases in other muscles were noted. The pattern of growth was quite different from that induced by growth hormone (63). The change in body com-

position was, as might be expected, an increase in water and protein in the usual proportions and a decrease in fat. There was no important alteration in the composition of several selected muscles. The steroid induced the same gain in weight in adrenalectomized rats as in intact animals. In hypophysectomized rats, the weight loss was reduced, and, in the female, the uterus was enlarged. It may be noted that the effects on organ and muscle weight were small, in accord with the relative weakness of methylandrostenediol as an androgen.

Dorfman & Shipley (202) give an exhaustive account of the effects of androgens on the concentration and activity of a variety of enzymes in several species of animals. In the case of the androgens it has generally been found that where the organ weight is affected by the hormone, the activity of certain enzymes is increased to about the same degree. Thus, Endahl & Kochakian (211) have found that the D-amino acid oxidase content of the kidney of the castrate mouse is increased as the organ weight is increased by a wide variety of androgens, but that the enzyme content as well as the weights of the kidney and liver of castrate rats and guinea pigs is not changed. This agrees with the work of van Bekkum & Kassenaar (212), except that the latter workers found an increase in D-amino acid oxidase of rat liver after testosterone. Kochakian & Endahl (213) also report that in castrate guinea pigs a number of androgens increase the transaminase activity and the weights of the temporal muscles to the same degree, but have no effect on the weight or the enzyme activity of kidney, heart or liver. In both castrate and thyroidectomized rats, the weight of heart and kidney is decreased; testosterone repairs these defects in the castrate but not in the thyroidectomized animal. In thyroidectomized rats, the liver arginase, transaminase, and ATP ase activities decrease in proportion to liver and body weight; testosterone is without effect on these activities (214). In accord with these findings is the observation that pretreatment of mice with testosterone increases the *in vitro* uptake of C^{14} -glycine into the kidney proteins but not into the proteins of other tissues (215).

The nitrogen-retaining effect of testosterone has been confirmed in the dog, in respect to the excretion of isotope after administration of N^{15} -glycine (57, 58). Sirek & Best (216) have found that testosterone does not have any nitrogen-retaining effect in the diabetic dog. Brown & Samuels (217), contrary to their earlier report, could not demonstrate nitrogen retention after a brief intravenous infusion of testosterone in man, although the same quantity, given over 24 hours, was effective. In careful balance studies in man, Borun, Geiger & Dahlberg (218) found that testosterone induced an early and disproportionate retention of water and particularly of potassium. In the period after treatment, water was lost but not nitrogen or electrolyte.

Sobel *et al.* (219) have reported on the effects of treatment of undersized children with small amounts of methyltestosterone for periods of six months. Although the hormone induced significant increases in rate of gain in height,

there was in most cases also a disproportionate increase in the rate of skeletal maturation.

The search for a nonandrogenic, protein-anabolic steroid continues. Not the least of the difficulties of this problem is the choice of satisfactory criteria for the quantitative estimation of the general anabolic effects as distinct from the purely androgenic effects. In nearly all of the work to date, the increase in weight of the muscle levator ani in the castrate rat has been used as a measure of the anabolic or "myotrophic" effect of a wide variety of steroids. There are reasons for doubting that this is a sound procedure, since in the rat, levator ani is unique even among the perineal muscles in the profound degree of atrophy which occurs after castration and in its sensitivity to androgen. On this basis, it could be classed as a component of the sexual apparatus. Eisenberg & Gordan (220) reported that in rats losing weight in consequence of hypo- or extreme hyperthyroidism, levator ani lost weight about in proportion to the general loss in body weight, and that in castrate rats treated with cortisone, the weight of the atrophied muscle possibly diminished somewhat further. This was taken to mean that since levator ani appeared to share in the general loss of body tissue in catabolic states, it would reliably reflect nonandrogenic influences, presumably anabolic as well as catabolic, on protein metabolism. It may be noted, however, that in the noncastrate rats in these conditions the seminal vesicles lost weight to an even greater degree than did levator ani. An intimate association of levator ani with the androgenic effect of a steroid is further indicated by the work of Nimni & Geiger (221), who showed that in castrate rats the weight of levator ani and of the seminal vesicles was increased by testosterone or by norethandrolone (17-ethyl, 19-nortestosterone) even when the animals were on a nitrogen-free diet. The conclusion was that levator ani is not suitable as an indicator of general protein-anabolic effects. Dorfman & Shipley [(202), pp. 122 to 23] have summarized data showing that different androgens may have disproportionate effects on different target organs. This suggests that the fact that a given steroid has a more marked effect on levator ani than it has, for example, on the prostate or seminal vesicles, need not indicate that it is any the less an androgenic substance.

In a comparison of the "myotrophic" effects of 32 different steroids with those of testosterone and its propionate, Hershberger, Shipley & Meyer (222) observed the relative effects of these substances on the weights of levator ani, the ventral prostate and the seminal vesicles of castrate rats. In this series 19-nortestosterone and some related compounds exhibited relatively greater effects on levator ani than on the prostate. The other steroids which had any effect at all, including methylandrostenediol, had substantially the same proportionate effects on these tissues as testosterone. In more recent studies by Barnes *et al.* (223, 224) and by Saunders & Drill (225), these observations on 19-nortestosterone have been confirmed and extended to additional derivatives, which exhibit similar disproportionate

effects on levator ani. It was found also, in agreement with Hershberger *et al.*, that methylandrostenediol does not differ qualitatively in its effects from testosterone, nor does androstanolone (androstan-17 β -ol-3-one) (224, 225). Stafford *et al.* (226) have attempted to determine the relative effects of 19-nortestosterone cyclopentylpropionate and of testosterone on nitrogen retention in castrate rats. They conclude that the effects of the two steroids are about equal, but their data reveal that the potency ratios might equally well have been 1:5, that is, in the ratio of their androgenic potencies in seminal vesicles (223, 224), as 1:1. Methylandrostenediol and androstanolone had relatively little effect on nitrogen retention. Thus, except for the effects on levator ani of which the interpretation is debatable, none of these steroids can be said certainly to have anabolic effects out of proportion to their androgenic properties.

In quite another direction, there is an interesting report by Landau *et al.* on the effects of progesterone in man (227). Daily doses of 50 to 100 mg. induced negative nitrogen balances up to 2.5 gm./day, without substantial effects on phosphate or electrolytes. The same effects of progesterone were seen in an Addisonian patient on supportive therapy, so that the role of the adrenal cortex hormones in this phenomenon seems at most permissive.

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MUSCLE¹

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If Engelhardt & Ljubimova's paper on myosin at the outbreak of World War II was the stimulus for the revival of interest in muscle physiology, this stimulus has endured. Muscle is no longer a research field exclusively of physiologists and biochemists. The phenomenon of muscular contraction now also intrigues the physicist and physical chemist. It has become a major field of interest in the new science of electron microscopy, as it was of the older histologists. An increasing number of clinicians are paying closer attention to the fundamental aspects of muscle physiology. The following selected list of 1956 publications indicates to some degree the activity and interest in the subject: an impressive monograph on energy coupling in muscular contraction by Aubert and a lengthy review on mechanical and thermal aspects of muscular contraction by the same author (13, 14); a critical review and summary on the chemistry of muscle contraction by Szent-Györgyi in a new monograph series (182); the whole September issue of the *British Medical Bulletin* devoted to papers on physiology of voluntary muscles; two symposia on muscle held at the XXth International Physiological Congress (36, 48a, 103, 118, 181, 199); two reviews on muscle published by *Physiological Reviews* in the same year (42, 147); a most interesting and provocative monograph on chemical physiology of excitation in muscle and nerve by Hayashi, introducing a new hypothesis (93); a theoretical review paper on the primary event in muscle action by Morales & Botts (136); an important monograph on tonus of skeletal muscle by Zhukov (207).

THE MOTOR UNIT

Territory of a motor unit.—A most useful electromyographic mapping of the territory of a motor unit has been provided by Buchthal, Guld & Rosenfalck (43). This study should dispel the persisting notion that one motor nerve fiber innervates exclusively an anatomically distinct group of closely packed muscle fibers such as a fasciculus. Having established that the fibers of the human brachial biceps pass uninterruptedly from tendon to tendon (41), the Danish group determined the dispersion of the muscle fibers of a motor unit from the recorded distribution of the spike potentials of a given motor unit over a cross section of this muscle. This was carried out by means of a multielectrode within a single needle cannula (1 mm. diameter) containing 12 leads distributed along a length of about 25 mm., thus overcoming

¹ The survey of literature pertaining to this review was completed in July, 1957. A considerable number of recent and current papers on muscle are not mentioned in this review because of its limited scope and the limitations of space.

to a major degree the uncertainties inherent in the single electrode displacement method. In a parallel study, the motor unit spikes from the same muscle, recorded in this case by a multielectrode whose 12 small leading-off surfaces were distributed over a length of only 2.5 mm., were subjected to volume conduction analysis (44). From this "analysis of the amplitude-distance relationship of the spike, from the spatial separation of different spikes of the motor unit and from histological evidence the spike was estimated to originate from the synchronized activity of *maximally* 30 muscle fibers" (44).

This number of fibers in the human brachial biceps can hardly represent a whole motor unit. On the other hand, the fibers of a single motor unit in this muscle are not widely distributed. They are localized and confined to an approximately circular region with an average diameter of five mm. However, such an area, they calculate, should contain about 10,000 fibers, or about ten motor units on the assumption of 1000 fibers per brachial biceps motor unit. They found experimentally up to six different motor units on the same lead during weak and moderate effort. The fibers of the overlapping motor units do not intermingle at random but those in each motor unit are distributed in small groups mutually separated by 0.3 to 1.0 mm. The spatially and temporally dispersed spikes recorded at different points in the motor unit originate from these groups or "subunits" (40) of closely packed fibers. Histological evidence for overlapping motor units in mammalian muscles, as mentioned by the authors, had been previously published (e.g., 70, 71, 89).

Multiple innervation.—The belief in single motor innervation, particularly in mammals, dates from the last century. Although repeatedly challenged at various intervals, this belief has been invested by some physiologists with unwarranted sanctity. In his summary of nearly a century of research on innervation of voluntary muscle, Tiegs emphasizes the difficulties in attempts to settle the problem histologically. "General experience with gold preparations gives little evidence of innervation by more than one ending, and this is the most that can be said" (189). The experimental evidence on frog muscle, up to the time his review was written (1953), was also discordant, and he concluded, "plainly the last word has not yet been said on the subject." In the same year appeared Kuffler & Vaughan Williams' papers, demonstrating that the small (gamma) efferent nerve fibers in frogs innervated a distinct set of skeletal fibers, the "slow" fiber system (125, 126). This aspect of their report seemed to have eliminated the possibility of one kind of multiple innervation, i.e., a skeletal fiber being supplied by both alpha and gamma efferent axons. However, they also provided experimental evidence in this paper of extensive polyneuronal gamma fiber innervation of these "slow" muscle fibers. They also called attention to the accumulating evidence for multiple innervation in frog and mammalian twitch muscles and for a dense innervation of intrafusal fibers within mammalian spindles (125).

The existence of multiple motor endings, from one or from several nerve fibers, at individual "twitch" muscle fibers in the frog and cat has now been

demonstrated unequivocally. The results of Hunt & Kuffler's simply conceived but elegantly executed experiments provide the conclusive evidence (102). The division of single nerve fibers in the frog, innervating widely separated regions of the muscle, was shown by axon reflex technique. Polyneuronal innervation was demonstrated in two ways. Up to 90 per cent tension overlap in the cat and nearly complete overlap in the frog was found by stimulation of various combinations of the lumbosacral ventral root outflow of the cat, including subdivisions of a single root, and ventral roots 8, 9, and 10 in the frog. Direct proof was obtained by intracellular recording. Identical action potentials were obtained from the same fiber after stimulation of separate efferent axons.

Hunt & Kuffler point out that the extent of multiple innervation is known only approximately, but on the basis of degree of tension overlap in the muscles tested, a large number of fibers must be innervated by more than one efferent fiber. The estimates of motor unit size, based on dividing total number of muscle fibers, or total tension developed, by the number of efferent nerve fibers reaching the muscle, will obviously have to be revised upwards. In addition to polyneuronal innervation it is also now known that about one-third of the efferent nerve fibers to the cat's hind limb muscles are gamma fibers, innervating intrafusal fibers of muscle spindles. Hunt & Kuffler consider the very brief latency of contractile activation as supporting the view that multiple excitation, if initiated synchronously at separated periods of a muscle fiber, may also facilitate the rate of activation. The full functional significance of multiple innervation is yet to be realized.

THE CONTRACTILE PROTEINS AND THE MOVING PARTS OF THE MYOFIBRIL

Actin, myosin, and other proteins within the myofibril.—Since the demonstration that "myosin" consisted of actin and myosin, these two proteins have been regarded as the "moving parts" of the muscle machine. To be sure, other proteins of the myofibril have been found. Tropomyosin, which will be considered in the smooth muscle section, was discovered early in the actomyosin era. Although a good deal of work has been carried out on this interesting structural protein since it was first described by Bailey (15, 16), its function is still uncertain. An unknown protein fraction extracted together with myosin, but which is not myosin, has been reported by Szent-Györgyi, Mazia & Szent-Györgyi (184). They suggested that this protein, of low molecular weight and low viscosity, might be the A substance responsible for the higher density of the anisotropic band, and called it therefore A-protein (182). Myosin is assumed to be distributed throughout the myofibril. Others have also called this new protein X-protein. Another fibrous muscle protein, delta protein, capable of forming a complex with myosin and of causing the dissociation of actomyosin, has been described (4, 26, 202). A specific role for delta protein has not been established, although it is believed that it may have some function in the contraction-relaxation

cycle. On the basis of electrophoretic mobility, delta protein does not appear to be the same as metamiosine, another new muscle protein described previously, which is distinguished from the known ones by solubility and electrophoretic mobility (158). [Useful tables summarizing chemical and physical characteristics of myosin, actin, and tropomyosin are provided by Bailey (17, 18). A recent table on the relative proportions of these proteins within the myofibril is provided by Perry (145, 146). A consideration of two other fractions of structural proteins, Y-protein and contractin, identified by electrophoresis, may be found in Dubuisson's monograph (62). A classification of muscle proteins may also be found in Hamoir's thesis (83).]

Actomyosin, the complex of actin and myosin, still accounts for about 80 per cent of the total structural proteins extracted from muscle. Above all, only the actomyosin particles, as in actomyosin thread models, have so far been demonstrated to possess contractile properties. Except for evidence in the recent single paper by Kafiani & Engelhardt (117), pure myosin or pure actin has not been found to be contractile by itself. Upon the addition of ATP, according to Kafiani & Engelhardt, myosin threads prepared from surface films by Hayashi's method (91) will contract anisodimensionally at a pH of 9. At pH 7 these threads will contract only in the presence of actin. Hayashi *et al.* carefully repeated these experiments but could not confirm them (92). They found that actin-free myosin lacked the ability to contract at either pH. The threads did develop a considerable amount of tension at pH 7.6 when they contained as little as 3.6 per cent actin. Some side-to-side interaction of actin and myosin, therefore, is still considered as inextricably associated with activation, development of tension, shortening, and relaxation of muscle. A clear, or even suggestive picture of the nature of the association and dissociation of these components in the actomyosin complex, however, has not as yet emerged. During most of the relatively recent actomyosin era the attention has been focused principally upon myosin as the component that "contracts." The postulated intramolecular changes, producing contraction, occurred in myosin. The presence of actin was indispensable but its role remained only vague. The association of actin and myosin is a precondition but the elementary act of contraction, as still held by Szent-Györgyi, is carried out by myosin (182, 183). By modifying the charge on myosin, as suggested by the work of Ashmarin (7), actin, at the side of myosin, may participate in triggering contraction but it is myosin which contracts (182, 183).

A concentration of attention on the role of actin began perhaps in 1953. In this same year during which Astbury stated that "the complete x-ray diagram of frog sartorius muscle has now been shown to be compounded of the separate diagrams of myosin and actin" (9), doubts were also raised as to whether there was any complex formation between actin and myosin (27, 190). Indeed, one year later, Chi & Tsao concluded that "there is no indication that actin and myosin interact in such a way as to form giant complexes or co-polymers" (53). In any case, if the significance, in molecular terms,

of Tsao's study of the behavior of labelled actin in the presence of myosin and ATP (190), raising doubts about the complex formation between actin and myosin, could not be fully assessed, his results nevertheless indicated for the first time "what appears to be an instantaneous change in the actin component actuated by myosin and ATP," as emphasized by Bailey (17). In the same year appeared the papers of Hasselbach (90) and Hanson & Huxley (86) reporting electron microscope observations of myofilaments in muscle after myosin extraction. Their conclusions that myosin is located in the A band of the sarcomere and that there are actin myofilaments were not new. Amberson *et al.* had already concluded in 1949 that myosin was located in the anisotropic segment (3). Szent-Györgyi had also assumed in 1951 that the myofilaments consist of actin and that the long very thin threads of myosin, invisible in electron microscope preparations, were located in the A band between the actin myofilaments (179). He states in this monograph that it is also H. H. Weber's opinion that myosin is located in the A band. An identification of myosin with the A substance and actin with the myofilaments was also suggested by Ashley *et al.* (6). The further conclusions of Hanson & Huxley, and their provisional model of the working parts of the muscle machine, however, reverses the "classical" roles of actin and myosin. Actin filaments in this model emerge as the principal moving components, with molecular changes, if any, primarily associated with the actin particles during contraction. Myosin is relegated practically to the role of ATPase. The single and longitudinally continuous skeletal framework of myofilaments traversing all bands of the sarcomere is supplanted by two distinct overlapping but continuous arrays of filaments.

Interdigitating model vs "classical" or modified classical model.—The new model is based upon three principal contentions. (a) There are two sets of myofilaments; the primary array of thick myosin filaments, 100 to 150 Å in diameter, are localized in the A band, and the thin actin filaments of about 40 Å in diameter extend continuously from each of the Z lines of a sarcomere through the I band and about half way into the A band, terminating at the edge of the H zone. There is thus an interdigitating arrangement between the two sets of filaments. (b) Myosin extraction of myofibrils removes the optically dense material of the A bands, the high birefringence, and the primary array of myofilaments. Actin, tropomyosin and other structural proteins left behind in the fibril after such extraction are presumed to be located in the thin filaments of the secondary array and in the material of unknown structure in the central H zone of the sarcomere. The remaining Z line might consist of "stroma" proteins. Additional extraction for actin removes the remains of the optically identifiable material except the Z lines. (c) Changes in length of the sarcomere after stretch or during contraction within physiological limits are due to alterations of the I band length only. The thin actin filaments are drawn out from within the array of the thick myosin filaments by stretch, extending the length of the I band without any extension in length of either set of filaments. The reverse process occurs during contrac-

tion, the actin filaments sliding between the parallel myosin filaments farther into the A band, filling up the H zone. Contraction to less than 90 per cent of rest length also involves shortening or coiling or both of the actin filaments but not the myosin filaments. A detailed account of this model is given by Hanson & Huxley (87) and summarized descriptions by A. F. Huxley (103, 104) and H. E. Huxley (110).

(a) The suggestion of two sets of parallel filaments was proposed by Huxley as an interpretation of his low-angle x-ray data on muscle (108). His electron micrographs of ultrathin cross sections, obtained by use of the Hodge-Huxley-Spiro microtome capable of cutting sections down to 200 Å (96, 97), Huxley felt, directly demonstrated the array of thin filaments, each of which was located between the individual thick filaments of the primary hexagonal array (109). The secondary array of dots in transverse section, also observed by Hodge in dipteran flight muscle and vertebrate skeletal muscle, was interpreted not as representing a distinct set of filaments but rather as one particular configuration of interstitial material localized in the A band (94, 95). Spiro has also observed thick and thin filaments in his longitudinal and oblique sections of rabbit psoas muscle but finds no evidence "whatsoever of interdigitation of the two types." The thick filaments, observed at rest and equilibrium length only at the H zone of the A band, are considered simply as thickenings or associations of several thin filaments (actin). The thin filaments in all of the A band are transformed into thick ones during contraction (171, 172). The association of filaments in pairs and an increase in filament diameter during contraction had also been observed earlier in isolated myofibrils by Ashley *et al.* (6). The thickening of the filaments in traversing the A band of relaxed fibrils (25, 161) was considered either as an association of some interfilamentous fixative-binding material in the A band with the filaments, or a greater fixative-binding capacity of the filaments themselves in the A band. A variation of diameter, as well as inner structure of individual myofilaments, in different cross bands, was also found by Sjöstrand & Anderson (169). The hollow or tubular nature of myofilaments, first reported by Farrant & Mercer for crab leg muscle (68), observed in rabbit muscle by Huxley (109) and Hodge (94), and in grasshopper leg muscle and blowfly wing muscle by Hodge (94, 95) also emphasizes the compound or composite nature of the myofilaments. Hodge considers that the evidence does not support Huxley's opinion that the tubular appearance is due to incomplete penetration of the stain. In Hodge's recent anatomical model of the fibril, therefore, the filament framework is considered as consisting of F-actin and the thickening in the A band as possibly due to a myosin or A-protein cortex around the actin filament (94). This anatomical design, in contrast to Huxley's, is of a single continuous set of myofilaments with variable diameters at different band levels and during contraction. Huxley's x-ray data, according to Spiro, can also be interpreted in this light.

Hodge's superb electron micrographs, particularly of transverse sections at very high magnification, clearly demonstrate transverse bridges between

the filaments, linking each myofilament to its six nearest neighbors in the hexagonal arrays (94, 95). The bridges are present at all levels of the sarcomere except possibly in the light H zone in the center of the A band. Others have also observed them (25, 150, 169). The composition of these bridges is unknown. It is not likely that they consist of either myosin or actin since both of these proteins are known to be aligned longitudinally. Hodge has suggested that they may consist of tropomyosin. He has inferred and Spiro (171) has definitely suggested that these structures may be responsible for the smaller dots in Huxley's electron micrographs which the latter interpreted to represent a smaller secondary set of filaments. This is unlikely in view of Huxley's most recent observations (111). The latest electron micrographs of ultra thin longitudinal sections of glycerol-extracted rabbit muscle by Huxley speak more convincingly for the reality of two kinds of filaments in the A band (111). The thin secondary filaments had not previously been observed in longitudinal sections, according to Huxley, because the sections were not thin enough. Huxley realizes, however, that the crucial questions concerning the continuity of his thin secondary filaments in the A band with those of the I band, and the discontinuity of the thick primary filaments in the A band with those in the I band, are not resolved by these electron micrographs. In the electron micrographs of longitudinal sections shown by Hodge, particularly of the blowfly wing muscles, the continuity of single filaments can clearly be traced from Z line to Z line (95). Similar micrographs of the housefly wing muscles are shown by Philpott & Szent-Györgyi (150). One can not accept Huxley's conclusion, therefore, that "the results which have been described above give full support to the 'sliding filament model' of striated muscle" (111).

(b) There is fairly general agreement concerning the existence of actin filaments, but there is uncertainty about the localization of myosin, if any, in the myofibril. The opinion that myosin is localized in the A band, as already mentioned, was emphasized by the demonstration by Hasselbach (90) and Hanson & Huxley (86, 87) that the A band of the myofibril disappears after selective myosin extraction of rabbit muscle and blowfly muscle (84). The demonstration that such extractions also remove another nonmyosin protein allowed an alternative explanation, that this A or X-protein is responsible for the density and birefringence of the A band and that myosin is also present in the I band (184). The suspicion that A-protein may contain pseudoglobulin, an inactive form of actin and possibly tropomyosin (113, 146), or the suggestion (147) that it may be related to Dubuisson's Y-protein (62), does not alter the fact that a protein, in addition to myosin, is extracted from myofibrils by such methods. The recent additional structural studies with the interference microscope and biochemical techniques by Huxley & Hanson strengthened the evidence for the concentration of myosin in the A band (88, 113). These authors are perfectly aware, however, that the results do not demonstrate the localization of all of the myosin in the A band. There can be no doubt that most, if not all, of the actin remains in the fibril after myosin extraction. But it is also clear from

Hasselbach and Hanson & Huxley's micrographs that when an unmistakable filamentous backbone is still discernible in the extracted myofibril the latter is not completely devoid of myosin. The myosin extraction experiments have not demonstrated the existence of separate myosin and actin filaments in the myofibril; neither have they rendered untenable the view that the filaments consist of actin threads coated with varying amounts of myosin at different levels. Szent-Györgyi, as near as one can judge, currently views the single continuous system of myofilaments as consisting of actomyosin, the actin and myosin of which are dissociated when muscle is relaxed and associated during contraction (181, 182). He has suggested in a footnote that the A-protein might be responsible for the thin or secondary array filaments (182).

(c) The interdigitation of myofilaments was first considered apparently simultaneously by two groups in England who were studying the changes in band pattern during stretch and contraction. A. F. Huxley & Niedergerke observed the changes in living frog fibers *in situ* by interference microscopy (105). H. E. Huxley & Hanson examined isolated myofibrils of glycerol-extracted rabbit psoas muscle in a phase-contrast system and in polarized light (87, 112). In contrast to the older observations with the ordinary light microscope, showing that both the I and A bands in frog and mammalian muscle participate in contraction, and that both bands are extensible (37, 38, 39, 99), they found that only the I band shortened during isotonic contraction and lengthened with stretch and that both I and A bands maintain a constant length during isometric contraction. This has been considered as particularly suggestive evidence in favor of the interdigitating model, the hypothesis that actin filaments are drawn into the A band between the myosin filaments during isotonic contraction and drawn out when the muscle is stretched. Until the sliding past each other of two distinct sets of discontinuous filaments is directly demonstrated, cross-striational changes described by Huxley & Niedergerke and Huxley & Hanson may also be interpreted as movement or shifts of interfilamentous material with the lengthening or shortening of a single continuous set of filaments. It is the molecular elasticity and molecular shortening within filaments of the classical model which is replaced by actin filaments sliding past stationary myosin filaments, neither of which change in length during either stretch or limited muscle shortening in the interdigitating model. Some or most observations by Huxley & Niedergerke and Huxley & Hanson on cross-striational changes during stretch and contraction have not been confirmed. Horvath reported in 1952 that the A band, as well as the I band, of the glycerol-extracted mammalian muscle lengthens during stretch (100). Philpott & Szent-Györgyi's electron micrographs of rabbit muscle strips show a lengthening of the whole A band, as well as the I band, during stretch (148). In addition they have called attention to the extension of the low density H zone in the A band during stretch as accounting for about 50 per cent of the total increase in sarcomere length. It is not simply an extension of the low density

length at the expense of the high density length in an otherwise unchanged total A compartment length, as believed by Hanson to be the case for both rabbit and blowfly muscles (84). The additional low density and elastic material in the H zone revealed by stretch was labelled "E band," and Philpott & Szent-Györgyi speculated that it might consist of tropomyosin. It may not correspond to Hill's series-elastic component, but Szent-Györgyi emphasizes that the "E band" material does constitute an elastically extensible, but not contractile, material continuous with the contractile portions of the myofilaments (182). Hanson & Huxley postulate, but have not demonstrated, an extensible "S filament" linking the discontinuous secondary actin filaments in the H zone (87). In their model, however, the thin actin filaments are pulled out of the A band by stretch, thereby presumably also stretching the "S filaments," lengthening the I band but leaving the A band length with its primary myosin filaments unaltered. The reinvestigation of the problem by Carlsen & Knappeis (52) on both living and fixed frog muscles with the polarized light microscope and on fixed fibers with the electron microscope also confirmed the earlier observations on the relatively high degree of extensibility of the anisotropic band in the living resting fiber.²

There seems to be fair, although not complete, agreement concerning the cross-striational changes during muscle shortening. Knappeis & Carlsen also found from their electron microscope studies of frog muscle that the main shortening during isotonic (afterload) contraction occurred in the I band (119). The Danish group emphasize, however, that the A band does shorten slightly when the whole sarcomere shortens from 5 to 35 per cent. The A band shortens considerably more upon greater contraction. Sjöstrand & Anderson consider their observations as supporting the conclusions of Knappeis & Carlsen (169). Horvath claims equal shortening in both bands of glycerol-extracted muscle (100). This might be due to the difference in relative shortening between fixed normal muscle and fixed glycerol-extracted muscle observed by Samosudova (163). Her electron micrographs also show gradations in degree of participation of A band in isotonic contraction, depending upon whether muscle was electrically stimulated before fixation, or whether stimulation was by fixative alone. There is no agreement on isometric contraction. The electron microscope studies of Knappeis & Carlsen (119) on frog muscles, with control of fixation artifacts, confirmed the older observations that the A band shortened at the expense of the I band during isometric contraction. Hodge also considers his phase contrast observations of A substance migration of glycerol-extracted blowfly myofibrils as in-

² In his forceful 1957 defense of the interdigitating model against criticism (111), Huxley does not mention or refer to Philpott & Szent-Györgyi's 1953 or Carlsen & Knappeis' 1955 papers.

In their 1955 paper (87), Hanson & Huxley consider Philpott and Szent-Györgyi's work as not free of artifact. Szent-Györgyi, on the other hand, can not understand why the English group failed to see the "E-band" (182).

compatible with the interdigitating model (94). Hanson failed to observe this migration to the Z line in another species of blowfly (84). The only internal shortening or movement visualized by the proponents of the sliding filament model during isometric contraction is a slight shift of the thin actin filaments into the A band at the expense of a series-elastic component, presumably at the Z line (87). The low density H zone is pictured as decreasing commensurately with the stretch of the series-elastic component at the Z line. Philpott & Szent-Györgyi claim, however, that their electron micrographs show just the opposite, that the internal shortening in isometric contraction occurs at the expense of the series-elastic component in the H zone, i.e., the dense material of the A band is narrowed as the H zone is stretched, the continuity of the myofilaments remaining unbroken (148).

In both models actin and myosin associate to produce contraction. In the interdigitating model myosin and actin are literally the moving parts. In the latter model the association is "mechanical," the moving parts retaining their dimensions during stretch and contraction within physiological range. The actin and myosin associate through linkages, the gross morphological outlines of which may be represented by the cross-bridges. The stationary myosin filament may pull the actin filament by means of these cross linkages, or preferably, according to Hanson & Huxley, the actin latches on and dissociates from the myosin in zipper-like fashion (87). It is not necessary in this model to postulate or search for molecular deformations to account for contraction. In the "classical" or modified classical model the filaments are both extensible and contractile, and the association of actin and myosin is more direct, intimate, and on a molecular level. If the filaments, which extend uninterruptedly across the entire sarcomere, consist only of actin, the shortening during contraction results from interaction with myosin which is present as a cortical layer around each filament. The cross-bridges are considered in this model as a system linking all the myofibrils, as well as a possible site of interaction, on a molecular level, of myosin and actin with each other and with the bridges. Instead of sliding of filaments there are shifts or migrations of interfilamentous material with shortening of the filaments during contraction which account for the observed cross-strational changes (95).

Components which are universal to all contractile or motile systems.—Cross-striation, with its bands, sub-bands, zones and lines, visible under the light microscope, represents distribution of material in the myofibril with different optical densities and different affinities for histological and electron stains. This is a feature of, and developmental specialization in, skeletal and cardiac muscles, but it is not specifically related to the mechanism of contraction. This specialization may possibly facilitate the interaction with ATP and account in part for the greater velocity of contraction in striated muscle, as compared to smooth muscle, actomyosin threads and muscle "models." The differences in composition between sarcoplasm and myofi-

brils also are not related to the banding in striated muscles. The structural proteins, accounting for about 65 per cent of the total (rabbit) muscle proteins, are in the myofibrils, whereas the myogens are in the sarcoplasm. ATP is utilized by actin and myosin in the myofibril but it is produced by the glycolytic and oxidative systems in the sarcoplasm. Most known muscle enzymes, as well as the oxidative systems of the mitochondria, are in the sarcoplasm (145). Only ATPase is part of the myofibrillar structure; in fact it was identified with myosin by Englehardt and Ljubimova 20 years ago. The submicroscopic morphological feature which seems to be universal to all contractile and motile organs is the organizational unit of the contractile material—the filament. It is present in the myofibrils of all smooth muscles so far examined by the electron microscope, as well as in striated muscles. Filaments have also been observed in cilia of protozoa and from a variety of ciliated organs in invertebrates and vertebrates including man, in protozoan and algae flagella, sperm tail and bacterial flagella (35, 69). In all, except bacterial flagella, there is also an intriguing fixed pattern of number of filaments per cilium or flagellum: 9 in the periphery and 2 in the center. The diameters of these filaments are remarkably similar to those of myofibrils. Bacterial flagella consist of single filaments and Astbury *et al.* regard them as "effectively monomolecular muscles" (12).

Actin and myosin, or actomyosin-like complexes constitute the common basis for all forms of contractility and motility. Flagellin, the name given to the characteristic protein from purified bacterial flagellar preparations (12), for example, may not be identical in chemical constitution with actomyosin (197, 198). But the x-ray diagram of flagellin establishes not only that it belongs to the keratin-myosin group of fibrous proteins but indicates also an axial period which is very little different from the axial period of muscle (10, 12). Also, such isolated flagella, or flagellar proteins, have been observed to contract upon the addition of ATP (60, 61). Similarly, glycerol-extracted flagella of spermatozoa and trypanosomes undulate in the presence of ATP. Such preparations are called flagella models since their movements correspond in all respects to the contraction of glycerol-extracted muscle fibers (muscle models) in the presence of ATP (98). In low concentrations ATP specifically accelerates ciliary movement (194), and the ATPase activity of flagellated single cells is concentrated in the motile organ (140, 187). This universality of a contractile protein complex is coupled with the ubiquity of ATP, the latter, as emphasized by Weber, constituting a link between metabolism and motility (196).

The role of ATP, energy transfer and contraction.—With the increasing prominence given to actin since 1953 and with the introduction of a non-classical concept of the moving parts of the muscle machine, some re-evaluations of or departures from the traditional view have also been made concerning the role of adenosinetriphosphate in the muscle machine. The enzymatic splitting of ATP, providing the free energy of hydrolysis for

immediate utilization was considered the primary event of muscular contraction.³ It has also been held possible that the dephosphorylation of ATP may be reversed by phosphate transfer from phosphocreatine. Physiologically, of course, excitation, or the muscle action potential is really the first event. The immediate effect of excitation, according to Szent-Györgyi's present outlook (182, 183), is the association of actin and myosin, which were dissociated before excitation. This association, perhaps, constitutes activation. The primary event, particularly in muscle models which do not possess an excitation mechanism, provides the energy for contraction of actomyosin. Whether ATP splitting is this primary event is not clear. Munch-Petersen reported positive results for single twitches in the tortoise muscle (137), and Lange for KCl and acetylcholine contractures (128). Mommaerts, on the other hand, not only criticized Munch-Petersen's calculations, but also reported no evidence for the fission of either ATP or phosphocreatine during single twitches of turtle muscle (132, 133, 134). Similarly, Fleckenstein *et al.* found no evidence for ATP, ADP, or phosphocreatine changes after 1 to 2 second tetanus of the frog rectus abdominis at 0°C. (73, 74). The latter reported the additional interesting observation, however, of an increase in inorganic P and an increase of an unidentified "fraction F," suggesting obviously the possibility of another source of "energy rich" phosphate. It has been emphasized that these negative results, in view of the technical problems involved in such experiments and the general acceptance of the positive findings for prolonged tetani, can not be accepted as yet as a final demonstration (42, 138, 147, 181, 182). Nevertheless, Mommaerts concluded his first notice of negative results with the statement, "the possibility of a new 'Revolution in Muscle Physiology' is clearly discernible" (132). In a public address in Europe, it is said, Professor V. A. Engelhardt commented that this was not a revolution but only a change in cabinet.

The failure to demonstrate the splitting of ATP as the primary event does not eliminate the role of this nucleotide as a link between metabolism and contraction. It really only questions the time of the ATP hydrolysis in the muscle working cycle. It may have a precursor, as Fleckenstein's experiments mentioned above suggest, or it may still serve as the immediate source of energy for contraction in a manner other than splitting, as suggested by a number of workers and most recently elaborated by Morales & Botts (136). If Szent-Györgyi marshals the evidence, particularly from experiments on glycerol-extracted muscle, in favor of the "potential energy of the

³ The loose usage by biologists since 1941 of terms like "energy rich" or "high energy" phosphate bonds, with implications of storage of energy in bonds and release of such energy by simple bond-dissociation, has been criticized by physical chemists, beginning, in this case, also in 1953 (78, 129, 144). As pointed out, such terms are misleading, as well as physically meaningless. Many who employ such terms of course are perfectly aware that they are unscientific, but because of "accepted" usage, continue to employ them. It might remove some confusion, it is argued, if this practice were abandoned in favor of less mystical physical and chemical terms.

P—O—P bond" of ATP as the energy source of contraction (181), Morales & Botts call attention to the several experimental conditions wherein myosin deformation and ATP splitting may be dissociated in the glycerol-extracted muscle model (30 to 34). In this light Morales & Botts propose again that ATP is the immediate source of energy, the primary event, but it is the noncovalent binding of ATP by myosin, a step prior to ATP splitting, which deforms the myosin system (135, 136). It is after the binding of ATP, presumably, that the latter becomes the substrate for the ATPase activity of myosin. But the free-energy drop involved in the ATP-myosin complex formation constitutes a large fraction of the total free-energy drop at the final state, i.e., ATP hydrolysis.

The demonstration by A. G. Szent-Györgyi & Borbiri that the mero-myosins, the sub-units of myosin which are arranged in series in the myosin molecule, consist of still smaller units, the protomyosins whose molecular weights are only about 4,500 (185), modifies somewhat the picture of the myosin "molecule" A. Szent-Györgyi now regards the myosin particle as an aggregate of protomyosins, rather than as a molecule (180, 183). In the theory proposed in his latest monograph, Szent-Györgyi considers the myosin particle in the resting muscle as stretched out, in an extended state. Morales & Botts hold a similar view. They visualize different mechanisms, however, whereby the particle is maintained in the extended state. Utilizing the entropic-electrostatic concept, developed particularly by polymer physicists, Morales & Botts propose that the mutual repulsion of the discrete charges fixed along the myosin chain constitutes the extensile force. If these charges are positive, supposing Mg^{++} adsorbed at the deformable regions of the myosin, the extended system will mechanically collapse, shorten to its equilibrium length, when the extensile charges are reduced by adsorption of the four negative charges of ATP. Relaxation, or extension, is reestablished by the removal or splitting of ATP. Szent-Györgyi suggests that the myosin aggregate is kept extended by the expanding water structures which the particles build around themselves. It is the splitting of ATP, in Szent-Györgyi's scheme, which collapses the water structures, rearranging the protomyosins into a shortened aggregate. Relaxation is brought about by rephosphorylation of the ADP to ATP.

The new monograph by Szent-Györgyi is devoted to the problem of energy transfer, or energy migration in living systems, particularly as it applies to muscle (183). One may recall that he introduced the migration concept into biology in 1941 (176), almost immediately after the phenomenon was discovered in physics. If there are conduction bands in inorganic crystals, i.e., the passage of an electron from some atom into an energy-level which spreads throughout the whole crystal, might there not be comparable energy bands in proteins? Jordan had already answered this in the affirmative in 1938 (116), and Szent-Györgyi called attention in 1946 and 1947 to the possibility that proteins may have an electronic structure analogous to that of semiconductors (177, 178). In his present monograph he proposes

that the "bond energy" of ATP molecules is transformed into a mobile and more active form of energy, i.e., electron excitation. To provide for the possibility of the excited electron being in the "triplet state" long enough to be an effective excitant for utilization in biological energy transmission, he also proposes water structures: water in an ordered state around the myosin particles. Such structures, he feels, may render triplet excitation probable and stable. An essentially identical suggestion, that water adsorbed on a protein may act as a proton semiconductor, as ice does, was made by Ril in his proposal of a proton mechanism for energy transfer in proteins (193). A third mechanism for energy transfer, resonance transfer, is considered by Shore & Pardee as the most probable, having much more general application (168). An exceptionally lucid review on the possibility of energy transfer in protein molecules has been written by Vladimirov & Konev (193).

The sequence of events leading to muscle contraction, as now visualized by Szent-Györgyi, may be schematically summarized in the following manner:

Excitation \rightarrow destroys balance of electrostatic forces which keep actin-myosin dissociated in resting muscle: ATP, linked to myosin, with its four negative charges, contributes to the maintenance of this balance \rightarrow actomyosin \rightarrow ATP now "activated" by myosin \rightarrow ATP split by ATPase to $ADP + \sim P \rightarrow$ "bond energy" $\sim P$ transformed to mobile excitation energy \rightarrow electronic excitation energy, transmitted to myosin molecule, may assume an unusually long-lived form (triplet excitation) by virtue of "liquid ice" or water structures which form a unique system with the myosin subunits, keeping the whole myosin chain stretched out when muscle is at rest \rightarrow collapse of water structures \rightarrow rearrangement of subunits (protomyosins) into shortened aggregate, i.e., contraction.

EXCITATION-CONTRACTION COUPLING

The mechanism whereby the action potential sweeping along the muscle fiber activates the contractile system is unknown. The problem of excitation-contraction coupling is usually introduced by the following question: How does the muscle impulse penetrate into the interior of the fiber, from 50 to 100 μ in diameter, and activate some 5,000 myofibrils within a single skeletal fiber? Whether the action potential triggers, "primes" or more directly initiates the events in the actomyosin system which lead to contraction, the event of activation itself can not be clearly defined as yet. It is clear enough that activation, an alteration in mechanical condition of the muscle fiber before external signs of development of tension, is characterized during the interval of a few msec by an initial rise in stiffness of the muscle, a resistance to stretch, a transient increase in optical transparency, an increase in pH, latency relaxation, and heat of activation. What is not clear is the nature of the alterations, initiated by excitation, which are responsible for the physical manifestations of the altered state of the contractile system, i.e., activation. If contraction is considered essentially as an interaction between actomyosin,

ATP and ions, does activation signify an association of actin and myosin, a mobilization of ions, like Ca, for the myosin-ATPase system, the binding or adsorption of ATP, the actual splitting of ATP, or possibly some combination of these factors? All theories of excitation-contraction coupling inevitably involve electrical forces. Only those proposed since 1955 will be considered in this review.

Electrochemical gradients.—Since there is normally a concentration gradient of Na and K across the muscle membrane, Fleckenstein proposes that the electrochemical events of excitation, involving diffusion of Na and K across the membrane, provide adequate free energy for contraction (72). This theory suffers from the major defect common to all schemes based on diffusion: diffusion is not rapid enough. It is also considered unlikely that chemical gradients, whatever role they may play in excitation, can supply the energy of contraction (42).

Z membrane as a transmitter of excitation to myofibril.—Tiegs entertained the possibility some years ago that the Z membrane may transmit the surface membrane excitation into the interior of the fiber (188). A. F. Huxley & Taylor consider that their observations of single sarcomere activation by microstimulation lend plausibility to this suggestion (104, 106, 107). They state that the localized cathodal stimulus was effective at the I but not the A band and that only the I band shortened even when the microelectrode was four μ in diameter at the tip. Stimulation with this size electrode shortened the I band for a distance of about 10 μ inward from the surface. In one of these very brief reports it is stated that the fibers were no longer excitable, i.e., propagated action potentials could no longer be elicited, at the time the above observations were made. It has been known for some time, of course, that localized twitch responses in excitable single fibers are obtained through microstimulation by virtue of the fact that the contractile mechanism is "directly" activated with stimuli which are not adequate for initiation of the propagated membrane response (76). Huxley and Taylor interpret their observations as "strong evidence that the influence of membrane depolarization is conveyed to the interior of the fiber by spread along some structure in the I band; from the anatomical situation, this must certainly be Krause's membrane (Z line)" (106). According to electron microscopists, however, the Z line in the middle of the I band does not "resemble a membrane in the usual sense, nor is there any clear continuity of the Z lines across the sarcoplasm between adjacent myofibrils" (155). Also, micro-injected oil droplets can freely move between sarcomeres of the living fiber (160).

Sarcoplasmic reticulum as an interfibrillar excitation conduction network.—Recent electron microscope observations on the fine structure of striated muscles have called attention to a sarcoplasmic reticulum (25, 64, 65, 152, 153, 162, 200) which is the equivalent of the endoplasmic reticulum found in all cells thus far examined except mature erythrocytes (141, 142, 143, 151, 154, 195). The electron microscope observations of myofilaments may not

have been immediately recognized as a rediscovery and confirmation of what Kölliker had described as "Faserchen" in 1888, but Bennett & Porter (25), Bennett (24) and Porter & Palade (155) promptly emphasized that a reticular structure within the sarcoplasm had been observed with the light microscope in the last century by Thin (186), Retzius (159), Cajal (51) and at the turn of the century by Veratti (192). A lucidly detailed account of this complex and intriguing tri-dimensional lattice, as it is intimately related to the myofibrils, in the sartorius and cardiac muscle of rat and in myotome fibers of *Amblystoma* larvae, has been provided by Porter & Palade (155). This network, as convincingly illustrated by electron micrographs, "consists of simple, membrane limited vesicles, tubules and cisternae associated in a continuous reticular structure which forms lace-like sleeves around the myofibrils" (155). The specific pattern within each sarcomere, particular units of the reticulum always coinciding with given segments of the myofibril it covers, is repeated in each sarcomere. Since this interfibrillar system is a closed one toward the cytoplasmic matrix, the continuity, which is principally across the fiber, and particularly at the H zone, involves both vesicular content and its enclosing membrane within the limits of each sarcomere.

Porter & Palade propose two possible functions for the sarcoplasmic reticulum. The latter may be considered as a sort of intracellular circulatory system, subserving perhaps special metabolic requirements of the myofibrils [see also (25)]. They also propose that the membrane limiting component of the reticulum might possess properties comparable to those of the sarcolemma and (in any case), as an obvious communicating network, might relay the action potential from the sarcolemma into the interior of the fiber. Realizing the implications of this, these authors are devising maneuvers for testing the suggestions. In attempting to account for the apparent synchronous response of the myofibrils within a fiber, Barer, in a review ten years ago, suggested that the sarcoplasm might possess special conducting properties (21). The sarcoplasmic reticulum thus provides the structural basis for such a conducting system which might also be excited by junctional potentials at the motor end-plate. Bennett (24) points out that Retzius had suggested an excitatory function for the reticulum in 1881.

Internal currents—Ca⁺⁺—activation linkage.—Ca is known to be a potent activator of the myosin-ATPase complex and there is suggestive evidence that Ca ions may be released from the immediate vicinity of the fiber membrane by excitation. Sandow had already considered the possible role of such liberated Ca⁺⁺ as a link between the muscle spike potential and activation (164). To move the liberated Ca from the surface into the interior, he suggested that the successive activation of contiguous myofibrils by a kind of exchange diffusion, with the possible help of electrostatic attraction, might be sufficiently more rapid than ordinary diffusion to bring about full activation within the known brief interval. Csapo & Suzuki now propose, instead, that the liberated Ca ions are moved by the internal currents which are known to flow along the interior of the fiber during excitation (59). The

movement of ionized Ca in this picture is only between Z lines of each sarcomere, about 2.5μ . Having demonstrated that skeletal muscle, rendered inexcitable by excess K, can be effectively activated by a longitudinal A.C. field, they consider that their analysis of degrees of shortening of different parts of such a whole nonpropagating muscle also suggests that effective internal currents are associated with the shortening produced by the external longitudinal field. In normal muscle, they argue, the internal longitudinal component of the action potential can effectively contribute to the activation process, in spite of the strong opinions to the contrary (173). Botts also considers Ca release as a triggering device for activation (29). The importance of Ca, with other ions, in actomyosin activation is unmistakable. Whether Ca is the key ion and link between excitation and activation in muscle, however, is yet to be demonstrated.

Core conduction and activation.—If the muscle fiber is considered as a core conductor, the problem of excitation-contraction coupling may be approached without invoking a mechanism for either rapid transport of ions or some substances from surface into the interior, or an appropriate anatomical pathway for penetration of action potential to the interior. An analysis of core conduction in muscle, comparable to the theoretical and experimental analysis for nerve by Lorente de Nó (130), is not available, but there is no reason to believe that muscle, like nerve, is not a core conductor. The axoplasm and the structures within it constitute the core of the nerve fiber. In the muscle fiber, the myofibrils and the myofilaments within them, as well as the sarcoplasm, must constitute the core. The significant aspect of this is that the major component of the core is also the contractile component of the muscle fiber. The lack of evidence for an anatomical membrane around the myofibril, comparable to the sarcolemma of the whole fiber, does not rule out the possibility that the surface of each myofibril is also polarized. Electric double layer dimensions are of the order of magnitude of atomic radii and a 1μ myofibril diameter may be some 10 times that of the smallest unmyelinated nerve fibers, which of course transmit nerve impulses (75). But a polarized surface around each myofibril would not alter the fact that myofibrils collectively constitute part of the core in each muscle fiber, as long as the entire content of the fiber is surrounded by a polarized membrane. It is inconceivable that all the transmembrane muscle fiber recordings which have been made with microelectrodes in the last decade represent electrical potentials of single myofibrils, rather than of single fibers.

If the contractile elements of the fiber constitute part of the core, as they must, it is not necessary to postulate any intermediary linkage between excitation and contraction. In his penetrating analysis of core conduction in nerve, Lorente de Nó has emphasized that the core can not be considered as an entity separate from or independent of the "membrane," that there is no definite demarkation between the two and that the core participates in electrotonic phenomena. The electrochemical events at the surface during excitation must simultaneously also involve the core, and actomyosin is in

the core of the muscle fiber. The nature of the alteration in the plastic actomyosin of the muscle at rest that "activates" it, as already stated, is unknown. Szent-Györgyi visualizes this possibility: "In resting muscle attractive and repulsive forces between actin and myosin are carefully balanced, 'on the razor's edge,' so that a small disturbance, as that brought about by 'excitation,' can upset it and bring actin and myosin-ATP together to form the actomyosin-ATP complex which, then goes over into the contracted state—" (182). It is not unreasonable to assume that the actomyosin can be so triggered or "activated" by the current flow in the core during excitation. The experiments of Csapo & Suzuki (59), as well as others on skeletal muscle, support such an assumption. The operating action potential mechanism, as already mentioned, can be bypassed with microstimulation and the contractile mechanism directly stimulated reversibly by electrical currents. The direct stimulation of the contractile system is facilitated if the conduction mechanism is blocked by Na lack or cocaine, or if the fiber is rendered inexcitable by depolarization with excess K. Indeed, the behavior of muscle under experimental conditions permitting "direct" stimulation of the contractile mechanism is considered by Csapo as a transition or link between living muscle and glycerol-extracted muscle models (54). If Ca ions play a role in activation, they do not have to be moved. Ca is not localized at the surface of the fiber membrane. It, as well as Mg and P is also present in the myofibrils (63), with a concentration periodicity of about 400 Å in each myofilament, and the disturbance at the core during excitation affects all myofibrils. The rate of muscle activation may reflect an intra- rather than an inter-myofibril activation rate. Activation of myofibrils by the normal excitatory process, as here postulated, is by virtue of the fact that the myofibrils constitute part of the core in the core conduction system of the muscle fiber.

SMOOTH MUSCLE, TROPOMYOSIN AND TONUS

Most considerations of smooth muscle are inevitably based on comparison with striated muscle. In his excellent analysis of uterine muscle function, Csapo concludes that the contractile characteristics of this smooth muscle, both at the molecular and cellular level, are in the main similar to those of skeletal muscle (56). Only the kinetics of reaction are different. The suggestion has also been made that behavior of smooth muscle is comparable to that of the A band in skeletal muscle at 0°C. (77).

The identity of the contractile proteins in smooth and skeletal muscles has been questioned in a report, already widely quoted, by Snellman & Tenow (170). Although Csapo and coworkers had extracted actomyosin from uterine muscle, identified actin and myosin as well as actomyosin in the Svedberg and Tiselius apparatus, and could duplicate with myometrium extractions the actomyosin thread models of skeletal muscles (56), Snellman and Tenow were unable to extract actomyosin in appreciable quantities from human and cow uterus. What they did extract was a complex of actin

and tropomyosin. The implications of this, concerning proteins which have contractile properties, are obviously important. Sheng & Tsao (167), as well as Csapo (56), however, seriously questioned the extraction methods used. Csapo had emphasized that modified extraction techniques have to be employed for the uterine muscle, and Kominz *et al.*, taking advantage of this information, succeeded in extracting the myosin-actin complex, as well as tropomyosin, in convincing quantities (121, 123). There are differences, however, between uterine and skeletal muscle actomyosin, as Csapo himself has stressed. ATPase activity of actomyosin from pregnant uteri, for example, has recently been found to be much lower than that of skeletal actomyosin (139).

Structure.—Smooth muscle cells are smaller than striated fibers and contain fewer myofibrils per fiber. A definite sarcolemma has recently been observed in uterine fibers, about 150 Å thick (131). Electron microscope studies have also revealed myofilaments in turtle and chicken intestinal muscle (201), in mammalian uterine muscle (56, 131), somewhat larger in diameter than in skeletal myofibrils; in longitudinal muscle coat of earthworm body wall, of about 250 Å in diameter (85) and in clam adductor muscle, of about 600 to 1000 Å in diameter (96). The filaments in smooth muscle do not appear to be regularly packed, as is so strikingly the case in striated muscle. While there is no differentiation between A and I bands in smooth muscle, Hanson described nonpenetrating transverse stripes bound to only one face of each fibril in the earthworm smooth muscle. These stripes are 300 to 500 Å wide, 1250 to 2500 Å apart, and she suggests that they may be comparable to the Z and M lines in striated muscle (85). Weinstein & Ralph have also observed an axial periodicity in smooth muscle myofilaments similar to the 400 Å periodicity in skeletal myofilaments (201).

Membrane potential.—Single smooth muscle fibers have also been pierced by small microelectrodes and the resting membrane potentials recorded: about 70 mV from smooth muscle of salamander stomach (79); about 60 mV from sphincter pupillae of isolated iris of albino rabbits (49) and isolated taenia coli of the guinea pig (45); 21 to 66 mV from isolated human, rabbit, guinea pig and cat uteri (203). The spontaneous spikes, superimposed in the records of the potential level from smooth muscle fibers contracting spontaneously, were surprisingly small, representing at most only 25 per cent of the resting membrane potential. In the more recent records from spontaneously discharging fibers of the pregnant guinea pig uterus *in situ*, obtained through flexibly mounted ultramicroelectrodes (205), the action potentials are larger than those previously recorded, with an occasional overshoot characteristic of skeletal fibers (204). The smaller resting membrane potentials, as compared to those in skeletal fibers, may in part be due to increased possibilities of current leakage attending electrode penetration of such relatively small fibers. If membrane properties vary with fiber circumference, as Håkansson suggests for skeletal fibers (80), the relatively smaller membrane potentials, as well as the slower conduction velocity in

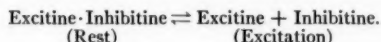
smooth fibers, may have an intrinsic basis. Similarity, the smaller action potentials, as compared to the resting membrane potentials, may not reflect purely technical problems. The frogs' "slow" striated fibers, regarded as occupying a position somewhere between striated "twitch" and smooth muscles, also have resting membrane potentials of about 60 mV (two-thirds of twitch fiber membrane potentials) and these slow fibers do not respond to electrical stimulation with propagated muscle impulses (125, 126).

Spontaneous rhythmicity.—This smooth muscle property, myogenic in origin, has been reinvestigated by Bülbring with intracellular electrodes. The influence of stretch and the correlation between tension, membrane potential and frequency of spike discharges has led her to the conclusion that smooth muscle of the intestine behaves like a sense organ. The spontaneous rhythmic contractions are the consequences of a contractile organ which has also the properties of a stretch receptor (46, 48). One can not help wondering whether it is the eventual complete dissociation of these two properties in smooth muscle which culminates in a skeletal muscle whose contractile functions are separated from the stretch receptor functions of the muscle spindle. Bülbring also noted the striking similarity in behavior between normal smooth muscle and skeletal muscle in Ca-deficient saline solutions (47). Since Adrian and Gelfan had demonstrated some years ago that Ca-deficient frogs' sartorius behaves like a stretch receptor (2), Bülbring *et al.* repeated these experiments and elegantly confirmed with intracellular electrodes the observations made with surface electrodes, and agreed with the interpretations and conclusions (50). It is not the general state of polarization of the Ca-deficient skeletal muscle but rather an unstable membrane potential that leads to spontaneous discharges. Spontaneous activity was observed when the membrane potential was raised by reducing also the K content of the solution. The initial slower potential changes, or prepotentials, which usually precede the spike discharges, and are observed with both surface and intracellular electrodes, are some indication of the membrane potential instability in such Ca-deficient fibers. The records, with surface and intracellular electrodes, showing the spike discharges taking off from the crests of the slower waves, or the prepotentials without action potentials, appear to be identical with the E_1 potentials of nerve fibers. This is the nomenclature of Lorente de Nó for the electronic component which, when it reaches a critical value, initiates the propagated impulse (130). Bülbring *et al.* point out the similarity to the prepotentials preceding spikes described for nerve by Arvanitaki (5) and to the records from crustacean sensory nerve cells with stretch receptor properties obtained by Eyzaguirre & Kuffler (66, 67).

In view of the fact that mechanical as well as chemical stimuli increase the rate of loss of radioactive K from normal smooth muscle (28), Bülbring *et al.* suggest that this mechanism might also be responsible for the responses of Ca-deficient skeletal muscle to stretch and to histamine (50). At any rate, an oscillating, fluctuating, "unstable" membrane potential, sensitive to stretch

and such substances as histamine, acetylcholine, and epinephrine is the normal state in spontaneously rhythmic smooth muscle.

According to Takashi Hayashi's interesting and new hypothesis of excitation in muscle and nerve, to which he was led through studies of spontaneous activity of Ca-deficient skeletal muscle, such spontaneous contractions are due to uncontrolled dissociation of two substances in muscle, excitine and inhibistine (93). The hypothesis may be summarized by the expression,



Ca lack interferes with the normal recombination of the two agents. From muscle extractions Hayashi eventually identified the initially hypothetical substances as carnosine (β -alanyl-1-histidine) and carnitine (betaine of γ -amino- β -hydroxybutyric acid), respectively, constituents of muscle which have been known since the beginning of this century, although no specific function had hitherto been ascribed to them. Since external application or intracellular injection of carnosine or carnitine, and combinations of the two, yielded the theoretically expected reactions, the hypothesis, as well as the experimental data, merits attention.

Uterine smooth muscle is a somewhat special case since the synthesis of the contractile system itself (actomyosin, ATP, phosphocreatine) is regulated by estrogen (56). Nevertheless the uterus, particularly the pregnant uterus, also exhibits spontaneous rhythmicity, is sensitive to stretch and tension, oxytocin, epinephrine, acetylcholine, and histamine. Assuming that polarization of excitable tissue is due to K and Na gradients across the membrane, Csapo is of the opinion that the pharmacological effects on the myometrium, including the estrogen-progesterone antagonism, are exerted through these electrochemical gradients (55 to 58).

Kinetics, tropomyosin, and tonus.—Conduction as well as contraction is slow in smooth muscle. If conduction were faster, say 2 to 3 M/sec, as it is in skeletal muscle, it would be utterly superfluous. All the evidence indicates that contraction in smooth muscle is intrinsically incapable of faster responses. It is hardly likely that the striations in skeletal muscles are responsible for its speed of contraction. Submaximal responses of contractile elements in a frog's sartorius single fiber "directly" stimulated, bypassing the conduction mechanism, can be as fast as normal twitches of the whole fiber (76), showing that the contractile mechanism is intrinsically capable of fast responses. Fast impulse propagation merely insures rapid activation of all contractile elements. On the other hand, actomyosin extracted from uterus reacts more slowly with ATP than skeletal actomyosin, although Csapo has stressed the importance of obtaining uterine actomyosin free of impurities before this is established as a fundamental property of smooth muscle actomyosin (56).

The differentiation of material which is responsible for striations is a superimposition on a contractile machine that is already relatively fast. We

now know that intrinsically slowly contracting fibers may also have striations. Kuffler & Vaughan Williams (125, 126) have demonstrated the existence in the frog of a distinct set of skeletal fibers, the "slow" fiber system, whose characteristics are qualitatively different from those of twitch muscles, and which are responsible for the "tonic" responses of muscles in this animal. Also, the prolonged contractions of a frog muscle immersed in KCl or acetylcholine solutions are due exclusively to the reaction of the slow components in such a muscle. There is some evidence that twitch and slow fibers may be distinguished histologically (127). But in the living muscle the slow fibers are distinguished under the microscope from the fast ones usually only on the basis of reaction to gamma or alpha motor nerve fiber stimulation (125, 126). Striations per se, therefore, do not confer speed to a muscle. If some of the striation bands in skeletal muscle, as suggested by the H zone in the middle of the A band, represent series-elastic elements, such striations may actually buffer the abruptness of onset and termination of twitches and tetani and thus the transmission of tension to the bony levers. Such a controlling mechanism is obviously superfluous for a smooth muscle system which is intrinsically slow in developing tension and even slower to relax.

The explanation of the difference in kinetics between slow and fast twitch fibers would be simple if the contractile proteins were different in the slow and fast fibers. But both smooth and skeletal muscles contain actomyosin, although not in the same concentrations. An alternative explanation, which has some merit and for which there is suggestive evidence, is that the actomyosin in smooth fibers is not identical with the actomyosin in skeletal fibers. This would require further explanation for the kinetics of the slow skeletal fiber system and of contractures exhibited by twitch fibers. A final proposal is that there are two contractile protein systems, closely related structurally, present in different proportions in all muscles. This would mean that all muscle responses are expressions in varying degrees of both "tonic" and "tetanic" components. The different rates of glycolysis during maintained tetanus, contracture and "tonus," as studied by Strelnina, are significant if confirmed (174). A dualistic contribution of tonic and tetanic components to the maintenance of posture, in which ATP and ATPase play important roles, is discussed by Postma (156, 157). Zhukov's monograph on the problem of tonus and skeletal muscles is most pertinent (207).

The "catch" or "holding" mechanism of molluscan bivalve muscles has been the classic example of tonus, maintaining contraction or extension for long periods of time. This type of tonus in bivalves, as recently shown by Hoyle & Lowy, is under nervous control (101), just as the tonic and holding activity of the "slow" skeletal fiber system in the frog is reflexly controlled (125, 126). One might add that the sphincter action of the circular muscles of the iris is also a holding action, reflexly controlled. Smooth muscle or tonic contractions are nevertheless slower than phasic or tetanic ones. The slowness of relaxation is even more pronounced and it is characteristic of the slow frog striated fibers (125, 126) as it is of the bivalves (1).

Ivanov and collaborators have presented evidence for the existence of a contractile protein, other than actomyosin, which is present in varying proportions along with actomyosin in vertebrate striated and smooth muscles and in invertebrate muscles (114, 115, 175). It is their contention that this unidentified protein is concerned with maintaining tonus whereas actomyosin is responsible for the twitch and tetanic contractions, and that there is a direct relationship between the type of activity of any given muscle and the concentration ratio of the two contractile proteins. Sheng & Tsao have suggested that tropomyosin may be related to this fundamental material which maintains tonus (167).

Since the original isolation of tropomyosin by Bailey from mammalian and fish striated muscles (16), this protein has been found, and isolated in a number of instances in crystalline form, in striated and smooth muscle of a considerable variety of vertebrate and invertebrate forms. The recent reports on tropomyosin have come from Japan (206), China (167, 191), England (20, 191), Belgium (83), and U. S. (120 to 123). The most significant aspect of this work on tropomyosin, as related to this discussion, is the difference in the molecular structure of tropomyosins from different sources. The differences are manifested by amino acid composition (120, 123), molecular size, shape, solubility, crystalline form, polymerizability, electrophoretic mobility, and the amount of associated ribonucleic acid (167, 191). Tropomyosins from mammalian striated and smooth muscle and from lower vertebrates are different. Greater differences are found among the invertebrate family of tropomyosins. Tsao *et al.* conclude that striated, smooth and cardiac muscles derived from different animals of the phylogenetic scale "exhibit delicate or marked species and functional differences" (191). Indeed, two different tropomyosins have been isolated from a single muscle (19, 20, 122). The paramyosin extracted from molluscan adductor muscles, considered as possibly contributing towards the holding activity of these muscles (23, 165), has now been shown to be the globular form of tropomyosin (20).

In his first paper on tropomyosin, Bailey suggested that this protein might be a subunit or precursor in the biogenesis of myosin. The similarity, on the basis of amino acid composition, between myosin and a mixture of equal parts of tropomyosin and actin impressed Kominz *et al.* as providing plausibility to Bailey's suggestion (120). This was further strengthened by finding a parallelism in amino acid composition between the tropomyosin and myosin of any given muscle among the different muscles so far examined (122, 123). Kominz believes that the free-SH groups in tropomyosin offer a mechanism for its incorporation into the myosin molecule.⁴ It is implied that the differences between actomyosins of different muscles might be due to the differences between the tropomyosin subunits. Tsao discusses the

⁴ The sections on -SH groups in Koshtojantz's monograph may be pertinent in this connection (124).

difficulties at the present time of reconciling this viewpoint with his and other evidence which fails to substantiate the proposition that tropomyosin is a subunit of myosin (191).

The problem of kinetics may be narrowed to two possibilities. Either there are "fast" and "slow" myosins, or there is a tonus protein which is not a myosin. These two general possibilities, however, are not mutually exclusive if we consider the following: (a) actin in some manner also participates in the deformation process which constitutes contraction; (b) the known subunits of myosin, the meromyosins, consist of still smaller units, the protomyosins; (c) there is at least partial similarity in amino acid composition and physicochemical properties between tropomyosin and myosin, and tropomyosin could be a prototype of myosin on a basis of molecular size; (d) other structural proteins besides actin, myosin, and tropomyosin, but whose function is still completely unknown, have been isolated from muscle; (e) how the structural proteins are linked together in the living muscle has not as yet been demonstrated. One may expect a *rapprochement* in the very near future, on this level, between U.S., China, and U.S.S.R.

MACROMOLECULAR PERIODICITY

It may be fitting to conclude this review with a note on macromolecular structure. A beaded appearance in many of the isolated myofilaments of fragmented and stained muscle with an axial periodicity of about 400 Å, was noted in the first electron microscope studies of striated muscle (82). This was soon demonstrated to be a regular repeating pattern, or fine macromolecular striation (63). This periodicity was first observed by x-ray diffraction of dried muscle (22a) and later confirmed in fresh muscle (108). The similar axial spacings shown by x-ray examination of actin films clearly pointed to an actin-rich, if not pure actin, system as giving rise to this periodicity observed in muscle by both methods (11, 8). In the recent x-ray diffraction analysis of the actin-rich "tinted" or striated portion of the *Venus* adductor muscle, Selby & Bear provided additional evidence that the 400 Å periodicity had its origin in an actin-containing system (166).

It was difficult to assess the functional significance of this observed axial periodicity. When Philpott & A. G. Szent-Györgyi (149) found that unstained light meromyosin crystals also showed a beautiful 400 Å periodicity under the electron microscope, a possible clue was provided for the actin-myosin interaction or association. As Astbury put it, at least a "dimensional correspondence" became apparent (10). Since Ca, Mg, and P residues seem to be localized in the fine transverse striations, spaced 400 Å apart in the relaxed myofibril, Hodge considers it likely that the dense lines in the meromyosin crystals may represent "bound" or accumulated salts between the ends of molecules (95). Further meaning was given to this axial periodicity by the demonstration that interfilamentous bridges in skeletal muscle, discussed earlier, were also spaced about 400 Å apart (95). In addition, these spacings seem to decrease as sarcomeres shorten (95).

Vertebrate smooth muscle also has an axial periodicity, varying from 322 to 743 Å, with 90 per cent of measurements in the range of 322 to 550 Å, as determined by the electron microscope (201). The axial spacings in invertebrate smooth muscle, determined by x-ray diffraction, is 720 ± 5 Å (22, 23). The latter periodicity originates from the paramyosin in the smooth portion of the bivalve adductor muscle (22, 23, 165). But, paramyosin, according to Bailey, is really tropomyosin (20). In the striated part of the adductor muscle in the same bivalve species the axial periodicity is 400 Å (166)! These finer or macromolecular striations, rather than the gross striations seen under the light microscope, are therefore promising for the understanding of structural protein linkages and the differences in kinetics between tetanus and "tonus" muscles.

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HEART^{1,2}

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MECHANICAL PERFORMANCE

The study of the mechanical activity of the heart has continued to excite interest, and has been pursued with resourcefulness. Rushmer (1) applied variable inductance and resistance gauges attached to the wall of the ventricle to monitor changes in internal and external left ventricular dimensions in the intact dog. Changes indicating an approach to a more spherical form occurred during the initial phase of ventricular systole. By the same technique, the so-called isometric phase in the right ventricle also appeared to be characterized by a change in shape [Anzola (2)]. Using similar methods Rushmer (3) obtained a continuous record of the relation between left intraventricular pressure and ventricular circumference which was displayed on a cathode ray oscilloscope as a series of closed loops which could be considered as an indicator of the work performed by the segment of myocardium circumscribed by the gauge. From such records it appeared that increased cardiac work secondary to exercise could be accompanied on one occasion predominantly by increased diastolic filling, and on another occasion in the same animal principally by augmented systolic ejection. Response to stimulation of the cardiac sympathetic nerves resembled that to exercise [Anzola & Rushmer (4)]. Buckley *et al.* (5) studied the effect of epinephrine and norepinephrine on left ventricular filling in a dog heart-lung preparation. At dose levels that produced a persistent inotropic and transient chronotropic response, changes in stroke output appeared to be related to ease and duration of filling.

By the application of small barium titanate crystals to send and receive sound signals across the heart, Rushmer *et al.* (6) studied changes in the left ventricular dimensions in the intact dog under various circumstances. In general, the left ventricle appeared to function near its maximum size, and remained fairly constant in dimensions from day to day in a given animal. Whether the increased stroke volume of exercise was achieved primarily by diastolic expansion or enhanced ejection depended on the ventricular diameter prevailing before exercise in the particular case.

¹ In line with the procedure followed by the reviewers of this subject in the previous year, the literature covered in the present chapter is limited to that published in the calendar year 1956, and was closed July, 1957.

² The following abbreviations have been used in this chapter: CoA coenzyme A; DPN diphosphopyridine nucleotide.

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Cotton & Bay (7) measured changes in cardiac contractile force by means of a strain gauge arch sewed to the ventricular wall in the dog. Mechanical stretching of the segment of myocardium between the points of attachment of the gauge, up to about a 50 per cent increase in length, was accompanied by an increase in contractile force. Further stretching diminished contractility.

Falholt (8) applied the dye dilution principle to the determination of residual ventricular volume having obtained good agreement between theory and experiment in a mechanical model. Cömet & Lagerlöf (9) presented a method for calculating left heart and pulmonary blood volumes from an analysis of indicator dilution curves. The method was also applied to the case of valvular lesions (10). Holt (11) estimated diastolic and stroke volume of the left ventricle of the dog by both a dye dilution and conductivity method, obtaining fair agreement between the two. In the anesthetized dog with closed chest, slightly less than half the diastolic volume, on the average, was ejected with each stroke.

The pressure cycles in the cardiac chambers and great vessels were re-examined in dog [Moscovitz & Wilder (12)] and man [Braunwald *et al.* (13)] with the aid of contemporary instrumentation. Rushmer *et al.* (14) recorded the movements of the mitral valve of the dog by cinefluorography with the aid of radio-opaque markers fixed to the valve cusps. Gobbato & Meda (15) estimated the duration of ventricular ejection from the carotid sphygmogram in normal humans at rest, and found it related to pulse rate and stroke output (calculated empirically), but apparently independent of arterial pressure. Van Harreveld & Russell (16) traced the postnatal development of the left-to-right atrial pressure gradient in cats, and found that it paralleled the increasing disparity between left and right ventricular mass.

A number of studies appeared documenting the development of a negative ventricular diastolic pressure (i.e., a pressure below that outside the ventricle) in hearts of various species [Bloom & Ferris (17), Bloom (18), Brecher (19), and Kraner & Ogden (20)].

The interesting phenomenon of post-extrasystolic potentiation was investigated in various isolated heart muscle preparations under a variety of circumstances [Penna & Garb (21), Hoffman *et al.* (22), and Scheider & Farah (23)]. The mechanism underlying this effect remains obscure.

Interest continued in the effects of temperature on cardiac performance, stimulated by use of hypothermia as an adjunct to cardiac surgery. Reissmann & Kapoor (24) found the maximum stroke volume attainable in the dog heart-lung preparation was little altered by hypothermia (above 22°). Maximum output declined with heart rate. The rise in atrial pressure with increased load occurred at lower work levels in hypothermia indicating a diminished work capacity. Badeer (25) and Reissmann & Van Citters (26) observed in the dog heart-lung that at constant work performance, a 10°C. temperature drop (from 36° to 37°C. initial temperature) was accompanied by an appreciable increase in calculated efficiency.

Hansen *et al.* (27) found that at 28° in the intact dog, ventricular work and oxygen utilization fell to one third of the control values, efficiency remaining unchanged. This is not in disagreement with the results observed in the heart-lung preparation, since in normothermia such a drastic reduction in cardiac work would be expected to be accompanied by a significant drop in mechanical efficiency. In contrast to the functional studies, areas of myocardial focal necrosis were reported following hypothermia in frogs [Sarajas (28)]. This observation merits further investigation. Other reports in this area included a study of the action current and mechanogram of frog heart muscle strips over the temperature range of 3° to 30°C. [Heintzen *et al.* (29)], a paper dealing with effects of rate on contractility of isolated turtle myocardium at different temperatures [Katzung & Farah (30)], and an investigation of the temperature range of spontaneous activity in the isolated atria of hibernating and nonhibernating mammals [Hirvonen (31)].

Some further (though all too scant) attention was given to the problem of work hypertrophy. Observations on the hypertrophy that accompanies the hypertension produced by constriction of the abdominal aorta in the rat were reported [Beznák (32) and Hügin & Verzár (33)]. Hypophysectomy prevented the hypertension and hypertrophy, and while their development could be initiated in the hypophysectomized animal by growth hormone, the activity of various preparations did not parallel the growth-promoting potency (32). A re-examination of the thesis that cardiac dilatation is an adequate stimulus for hypertrophy yielded negative results [Stickney *et al.* (34)].

Concerning the relationship between mechanical performance and metabolic factors, Winbury (35) studied the effect of glucose on the contractility of cat papillary muscle during and after anoxia of varying duration. The presence of glucose facilitated recovery from prolonged anoxia (in excess of 15 min.), a finding prompting the suggestion that the energy derived from anaerobic glycolysis, while inadequate to maintain contractile activity of the heart, is adequate to preserve the functional integrity of the contractile system. Webb & Hollander (36) compared the effects of metabolic depression due to anoxia and dinitrophenol on contractility and action potential of the rat atrium, and found them similar. Blain *et al.* (37) measured myocardial extraction of oxygen and numerous substrates in patients with congestive failure, and found no striking deviations from the normal. It was concluded that the impaired mechanical performance of the heart resulted from a failure in the conversion of chemical energy to work, and that some change in the contractile proteins might constitute the primary defect. A similar view was expressed and documented by Olson (38). However, significant differences in substrate uptake may result in insignificant changes in the arteriovenous difference, and even if we assume equality of substrate uptake by the normal and failing heart, the ultimate metabolic fate of energy-yielding fuels may differ in the two, involving, perhaps an uncoupling of oxidative phosphorylation beyond the substrate level. While the concept of a defect in the conver-

sion of chemical energy to work as the cause of myocardial failure is an attractive one and has enjoyed wide adherence, present evidence will not sustain a firm decision between this thesis and that of a defect in the transformation of the free energy of substrate oxidations to a biologically useful form.

Katz (39) and Laurent *et al.* (40) described a spontaneous and reversible increase in coronary flow and decrease in oxygen utilization in hearts working at excessively high rates or under heavy load. This was interpreted as a metabolic adaptation to stress. The elucidation of this phenomenon in tangible chemical terms will represent an important contribution.

Ullrick & Whitehorn (41) reported the development of a respirometer in which electrical and mechanical events as well as gas metabolism could be studied in small strips of cardiac muscle.

A number of studies concerned with cardiac function under abnormal circumstances may be mentioned briefly. Rose *et al.* (42) by inducing ventricular fibrillation and substituting an extracorporeal pump for the left ventricle were able to study circulatory dynamics in the presence of a nonfunctional right ventricle. Brostoff & Rodbard (43) studied the hemodynamics of ventricular septal defects in a model system. Haring *et al.* (44) recorded the left atrial electrokymogram and pressure, and the pulmonary wedge pressure in surgically induced mitral stenosis and insufficiency. Burger & Van Brummelen (45) examined the relations between flow and pressure across a stenosis in models. Friedman *et al.* (46) described a method for determining the presence of mitral insufficiency, involving the injection of a solution of NaCl into the left ventricle and the detection of reflux by a conductivity cell in the left atrium. The method should also detect electrolyte that may enter the left atrium by mixing during diastolic filling, and the results suggest that this may actually occur. Hawthorne *et al.* (47) produced varying degrees of congestive failure in dogs by combining atrial septal defects with aortic valvulectomy and renal artery occlusion. Omission of the septal defect led to rapid death with acute pulmonary edema (48). Clemenson (49) reviewed the effects of blast injury on cardiovascular function.

The anatomy and physiology of ventricular function were ably reviewed by Rushmer (50).

While considerable progress has been made in the study of the mechanical activity of the heart, particularly *in vivo*, knowledge of the relationship between work and energy release in heart is still rudimentary in comparison with what is known about skeletal muscle. This is probably due to the unsuitability of heart muscle for accurate myothermic studies. Other ways will have to be found in order that the attack on this important problem may be renewed.

CARDIAC OUTPUT

The study of cardiac output has provided material for numerous reports. The application of bromsulphalein [Wassén (51)] and of an ear-oximeter [Kaufman & Hegglin (52)] to the dye-dilution technique was described, and

the present status of the method reviewed [Dow (53)]. Crane *et al.* (54) used a radioactive indicator with continuous recording to study flow in a circulation model. Flowmeter and indicator results were in excellent agreement. Sapirstein (55) studied the organ distribution of K^{42} in the rat following a single intravenous injection, as an indicator of the fraction of the total cardiac output flowing through each organ.

Cardiac output was measured in dogs under a variety of anesthetic agents [Nash *et al.* (56), and Greisheimer *et al.* (57)], in the presence of hypervolemic and normovolemic anemia [Fowler *et al.* (58)], of bilateral carotid occlusion [Brind *et al.* (59)], and in exercise [Leusen *et al.* (60)]. de Arellano *et al.* (61) applied the indicator-dilution method to a study of pulmonary blood flow in patients with central arteriovenous shunts. Silver *et al.* (62) examined flow patterns through experimental atrial septal defects in dogs, and found evidence for incomplete mixing in both atria.

A good deal of attention was devoted to the theoretical and technical aspects of ballistocardiography (63 to 74) with evident progress.

CORONARY CIRCULATION

Various factors that affect coronary flow have been studied in the dog heart isolated *in situ* [Laurent *et al.* (40), and Allela *et al.* (75)], and in the intact dog [Hackel & Clowes (76)], and have been reviewed [Gregg & Sabiston (77), and Katz (39)]. Agreement seems fairly general that metabolic events in the myocardium exert the predominant influence on the caliber of the coronary vessels, and hence the flow. The nature of the presumed chemical mediator in this relationship remains to be defined. Along this line, Wolf & Berne (78) examined the coronary vasodilator properties of a number of purine and pyrimidine derivatives. ATP and ADP were the most active of the compounds tested. While they are both polyelectrolytes and presumed not to penetrate cell membranes readily, neither one can be excluded from candidacy for the role of chemical mediator on the basis of available information. Denison *et al.* (79) found in dogs that epinephrine, norepinephrine, and isopropylarterenol, which have been reported to act differently in various vascular beds, produced a consistent dilatation in the coronary vessels (and an increase in myocardial contractility).

Coronary blood flow was found to lie within normal limits in patients with pulmonary emphysema and cor pulmonale [Rose & Hoffman (80)] and to be elevated in thyrotoxicosis in humans [Rowe *et al.* (81), and Leight *et al.* (82)]. Normal values were restored by thyroidectomy or treatment with I^{131} (81). Rinzler *et al.* (83) described a method for the detection of coronary atherosclerosis in rabbits based on electrocardiographic changes induced by intravenous ergonovine.

Additional documentation has been added to the growing literature on the altered chemical and enzymatic composition of myocardium and plasma following acute coronary occlusion in men and animals (84 to 89); and further work on the problem of increasing the blood supply to the heart by

surgical procedures has been reported (90 to 92). Blatteis & Horvath (93) recorded pressure curves from a depth of one to two cm. in the coronary sinus of the dog, and found variable contours, with pressures ranging from 2 to 6 mm. Hg. Hansen *et al.* (27) used krypton⁸⁵ for the determination of coronary flow by the foreign gas method in dogs. Sapirstein & Ogden (94) in a discussion of the limitations of the nitrous oxide method for the measurement of regional blood flow called attention to the errors that may result if the assumption of concentration equilibrium between the organ and its venous blood does not in fact obtain.

BIOCHEMISTRY OF HEART MUSCLE

The heart continues to offer fertile ground for the study of the intermediary metabolism of various substrates. Stern *et al.* (95, 96) investigated the breakdown and synthesis of β -keto fatty acids in heart, and reported progress in the isolation of enzymes which catalyze the activation and cleavage (as well as synthesis) of acetoacetate. Activation is accomplished by the reversible transfer of CoA from succinyl-CoA to acetoacetate. The abundance of this enzymatic activity probably accounts for the rapid rate with which the heart utilizes acetoacetate, as compared with other tissues. Bachhawat *et al.* (97) presented evidence for acetoacetate formation from isovalerate (an intermediate in leucine metabolism) in heart (and liver). The differences between heart and liver with respect to ketone body metabolism are undoubtedly a reflection of quantitative differences in enzyme constitution. Meyer & Bow (98) infused C^{14} -labeled acetoacetate into an isolated dog heart preparation. Label appeared in malate and succinate predominantly in the carboxyl positions, a result interpreted as consistent with current concepts of acetoacetate cleavage, and oxidation via the Krebs cycle. Administration of C^{14} -labeled acetoacetate to intact rats (99) failed to label the cardiac glycogen, although cardiac glycogen is characteristically elevated in ketosis or following infusions of acetoacetate. It seems clear that acetoacetate carbon is not incorporated into cardiac glycogen to an appreciable extent. Because of the rapid rate of acetoacetate oxidation in heart, elevated plasma levels may exert a carbohydrate-sparing effect, hence the increment in cardiac glycogen. Cavert & Johnson (100) studied the rate of conversion of various C^{13} -labeled short-chain fatty acids to CO_2 in an isolated dog heart preparation. Isotope could not be detected in the glycogen.

The metabolism by heart of carbohydrate and carbohydrate intermediates was investigated in a variety of ways. Montgomery & Webb (101) presented evidence for the oxidation of pyruvate via the Krebs cycle in a washed mitochondrial suspension from rat heart. While all the classical criteria for the operation of the cycle were not applied, it seems reasonable to conclude that the vigorous oxidative metabolism of pyruvate exhibited by this preparation occurs largely via the cycle. The mechanism by which parapyruvate (a common impurity in samples of pyruvate) inhibits oxidative metabolism was investigated in mitochondrial and enzyme preparations from rat and

pig heart (102, 103). Haugaard & Itskovitz (104) studied the oxidation of various hexoses by a rat heart homogenate. Co-factor requirements were defined. Glucose, fructose, and mannose were oxidized at equal rates, galactose less rapidly. A number of pentoses were tested and found inert. Fisher & Lindsay (105) studied the action of insulin in the perfused rat heart, where the primary effect of the hormone appeared to be on the rate of entrance of glucose and related sugars into the cell. Penetration of the cell membrane in heart showed a lesser insulin-dependence than in skeletal muscle. Galactose was not utilized by the intact rat heart. Allela *et al.* (75), using a dog heart preparation isolated *in situ*, measured glucose and lactate uptake. Disappearance of these exogenous substrates accounted for only a part of the total oxygen utilization, as usually found in this type of study. Leight *et al.* (82) measured uptake of glucose, lactate, and pyruvate by the heart in normal and hyperthyroid humans. A wide scatter occurred in both groups, but no difference between the two was evident, although coronary flow and oxygen utilization were elevated in the hyperthyroid individuals. In both groups substrate disappearance accounted for less than half the observed oxygen uptake. Cavert & Johnson (106) studied the conversion of carboxyl- C^{13} and α,β - C^{13} -labeled lactate to carbon dioxide by the isolated dog heart. Rate of conversion was more rapid for the carboxyl group, while oxidation to carbon dioxide lagged behind disappearance as determined chemically. (It seems clear that the significance of substrate uptake as customarily measured is subject to an undetermined element of uncertainty.) Isotope could not be detected in the glycogen, raising a question as to the importance of the glycolytic pathway in the resynthesis of cardiac glycogen. Buzard *et al.* (107) observed a rapid loss of glycogen without a corresponding accumulation of lactate in heart slices in the absence of oxygen, and suggested the possibility of a pathway for lactate removal other than the classical DPN-linked lactic dehydrogenase. A similar proposal has been advanced recently by others, based on the persistent uptake of lactate by the dog heart under hypoxia *in vivo*, and the reported occurrence of a DPN-independent lactic oxidase in other tissues and in some micro-organisms. However there is as yet no conclusive evidence for the existence in heart of a significant alternate pathway for lactate oxidation. Bloom (108) did not detect appreciable glycogen breakdown in the excised beating rat heart in anoxia unless work was done, and suggested that anoxia *per se* was not the prime cause of glycogenolysis in the working heart (a point which may be argued).

Green & co-workers (109 to 111) studied electron transport and coupled phosphorylation in submitochondrial particles from beef heart. Oxidative phosphorylation in mitochondrial (and submitochondrial) preparations from heart, as compared with other tissues, appears to be remarkably stable. Dianzani & Scurio (112) observed morphological changes in mitochondria from rat heart and other tissues following exposure to chemical agents that induce uncoupling of oxidative phosphorylation.

Individual enzymes from heart, particularly of the electron transport chain and Krebs cycle, continued to receive active attention. Among the topics dealt with may be noted the physical constants of highly purified cytochrome C [Atlas & Farber (113)], oxidation-reduction reactions of cytochrome C [Mee & Stein (114), Smith & Conrad (115), and Henderson & Rawlinson (116)], the effect of various inhibitors on the cytochrome systems of heart muscle [Lightbown & Jackson (117)], and the cytochrome C content of human heart and skeletal muscle [Börck (118) Börck (119)] reviewed the major heme derivatives of mammalian heart and skeletal muscle, and their physiological significance. Studies of various aspects of the enzymology of myocardial isocitric dehydrogenase [Moyle & Dixon (120), and Moyle (121)], malic dehydrogenase [Wolfe & Neilands (122), and Graves *et al.* (123)], aconitase [Herr *et al.* (124)], succinic oxidase [Thorn (125), and Tookey & Balls (126)], lactic dehydrogenase [Takenaka & Schwert (127)], tartaric oxidase [Kun & Hernandez (128)], and phosphorylase [Rall *et al.* (129)] have been reported. Hearn & Wainio (130) produced ventricular hypertrophy in rats exercised by swimming but found no change in the succinic dehydrogenase activity of the heart or skeletal muscle. They concluded that the normal concentration is adequate to cope with the stress of moderate exercise.

That altered functional demand can lead to changes in the chemical constitution of the myocardium was further documented by the report of an elevated level of cytochrome C in the right ventricle in pulmonary hypertension (118), and the finding of an increased myoglobin content in heart (and skeletal muscle) of rats acclimatized to high altitude [Vaughan & Pace (131)]. The importance of bringing the animals to the fully acclimatized state was pointed out.

Reports concerned with the chemical composition of the myocardium under various circumstances include a comparison of atrial and ventricular content of sodium, potassium, phosphate, phosphocreatine, adenosine polyphosphates, glycogen, and lactate [Mulder *et al.* (132)], a study of adenine nucleotide levels in anoxic arrest [Burdette (133)], and an analysis of the flavin enzyme and coenzyme content of heart (and other tissues) of rats on diets containing various levels of riboflavin [Burch *et al.* (134)].

Walter *et al.* (135) noted a preferential uptake of label by embryonic heart when a homogenate of adult chicken heart containing S³⁵-labeled protein was injected into the chick embryo. Myocardial necrosis in severe choline deficiency in rats was described by Wilgram *et al.* (136). Carlson *et al.* (137) found the oxygen uptake of rabbit heart mitochondria inhibited by Streptolysin O, an effect which appeared to be due to the presence of a bacterial diphosphopyridine nucleotidase in the preparation. Isolated perfused hearts exhibited loss of contractility when exposed to Streptolysin O [Kellner *et al.* (138)].

Dettli & Bing (139, 140) studied the contractility of threads and bands of actomyosin from dog heart. Benson *et al.* (141) found an apparent decrease

in actomyosin in hearts of dogs in congestive failure induced by valvular lesions. Flory (142), in a review of the role of crystallization in polymers and proteins, presented a treatment of muscle shortening. Buchtal *et al.* (143) contributed a review on the mechanical and chemical events in muscular contraction.

It may be anticipated that the relationship between the physiological function and biochemical properties of the myocardium will provide an increasingly fruitful field for investigation.

EFFECTS OF NERVES AND HUMORAL AGENTS

Randall & Rohse (144) found the elevated arterial pressure following electrical stimulation of the thoracic sympathetic trunks in the open-chested dog was attributable primarily to augmentation of the ventricular beat rather than increased rate. Stimulation of the left sympathetic produced ventricular augmentation without a rate effect, while the right gave both. Anzola & Rushmer (4) observed that the cardiac response to electrical stimulation of the sympathetic cardiac nerves and to exercise was similar in the intact dog. This was characterized by reduction in both diastolic and systolic volumes, elevated systolic pressure, and tachycardia. A comparable pressure rise due to epinephrine was accompanied by bradycardia. Folkow *et al.* (145) reported that stimulation of the cardiac sympathetic nerves had a much more pronounced rate effect than stimulation via the adrenals. Pre-ganglionic sympathetic innervation was studied in the cat heart by means of electrical stimulation and various blocking agents [Szentiványi & Kiss (146)].

Denison *et al.* (79) observed coronary dilatation and myocardial stimulation following administration of epinephrine, norepinephrine, and isopropylarterenol in the open-chested dog. Garb *et al.* (147) studied the rate and amplitude effects of the same three agents in the atria of rat, guinea pig, rabbit, and cat. Isopropylarterenol exhibited much higher potency in all species. Amplitude and rate effects were more marked at 29° than at 37°C. [Garb & Penna (148)]. In the denervated cat heart the rate effects of epinephrine and norepinephrine were similar [Weisberg & Griffith (149)]. However, norepinephrine appeared to be more active than epinephrine in eliciting ventricular ectopic rhythms in the anesthetized cat, as well as automatic contractile activity in the isolated cat papillary muscle [Hutcheon (150)]. Hild & Herz (151) reported an increased isometric pressure and stroke volume and decreased diastolic volume following epinephrine administration in the isolated cat heart. The contraction and relaxation phase were both shortened [Herz (152)]. The effect of epinephrine and norepinephrine on filling of the left ventricle in a dog heart-lung preparation was described by Buckley *et al.* (5).

Goodall & Kirshner (153) found the norepinephrine content of the dog heart sharply reduced by removal of the right cervical cardiac ganglion (extirpation of other cardiac ganglia had no effect). In sheep, surgical ablation of both the right cervical and thoracic ganglia resulted in a similar

change. Von Euler (154) could observe no change in the epinephrine or norepinephrine content of the heart following infusion of these agents in the cat; an increase has been reported in the dog (155). Conflicting reports on the total catechol amine content of human hearts in cardiovascular-renal disease appeared (156, 157). A method for total tissue catechol amine content was described [Bloodworth & Von Haam (158)]. Ellis (159) reviewed the metabolic effects of epinephrine and related amines.

McEwen (160) described an innervated rabbit Langendorff and isolated atrial preparation, in which the effects of vagal stimulation were studied. The response to vagal stimulation could be elicited over a longer period of time in the isolated atria (more than forty hours) than in the whole heart preparation. The influence of a number of drugs on the vagal response was examined. Stade & Weiss (161) gave a mathematical treatment of the time-course of the vagal chronotropic effect in the rabbit heart, based on diffusion laws and an idealized concept of the humoral transmission mechanism.

Moulder & Thompson (162) observed a prolongation by hypothermia (24° to 27°C.) of the slowing induced by intracoronary infusion of acetylcholine. Fänge *et al.* (163) described a method for studying single cells and cell-clusters of embryonic chick heart in culture. The spontaneous rhythm of atrial cells was found to be much more sensitive to acetylcholine inhibition than that of ventricular cells.

Marshall & Vaughn Williams (164) abolished the electrical and mechanical activity of the isolated rabbit atrium by cooling (14° to 20°C.), and found that both could be restored by acetylcholine at a dosage level (10^{-6} to 10^{-8} gm. per ml.) which was without effect at 30°C. Trautwein *et al.* (165) studied the effects of acetylcholine on the electrical characteristics of isolated muscle strands from frog atria. Leveque (166) reported the production of atrial fibrillation by the infusion of acetylcholine in hyperthyroid dogs. Burn *et al.* (167) found evidence for the liberation of acetylcholine in the dog heart-lung preparation, possibly from nonnervous tissue. Beznák (168) reported an increase in the choline ester content of hearts from thiamine-deficient rats.

Zingoni (169) described an apparatus for perfusion of the embryonic chick heart, and the effects of epinephrine and acetylcholine on rate and amplitude in this preparation (170). In the 68 to 70 hr. embryo (in which nerves have not yet reached the heart) the response to both agents was similar to that in the adult heart. Lynch & Essex (171) produced fatigue to vagal stimulation in an innervated Langendorff preparation of the guinea pig heart, and found that infusion of epinephrine restored the vagal effect. Effects of vagal and sympathetic stimulation [Hutter & Trautwein (172)], and of epinephrine and acetylcholine [Webb & Hollander (173), and West *et al.* (174)] on the membrane potentials of heart muscle fibers were reported. Day (175) observed the release of both acetylcholine- and epinephrine-like materials by the isolated rabbit heart.

Augustinsson *et al.* (176) compared the autonomic innervation of hagfish

and lamprey hearts. While both hearts contain large amounts of epinephrine and norepinephrine, they are little influenced by the administration of either agent. The lamprey heart receives vagal fibers and responds to vagal stimulation by acceleration followed by slowing. The hagfish heart has no nervous elements, and its rate is unaffected by vagal stimulation. Other interesting differences were pointed out. Jullien & Ripplinger (177) studied the effects of acetylcholine and the stimulation of vagus-type cardiac nerves on the hearts of molluscs and fish. Evidence was found for the liberation by the hearts of these animals of an acetylcholine-like substance, which appeared in greater amount with increased cardiac work. The effects of epinephrine and acetylcholine on the heart rate of *Anopheles* [Jones (178)], and of extrinsic cardiac nerves and autonomic humoral agents on the heart of *Helix adspersa* [Corda (179)], and Peruzzi & Corda (180)] have been reported.

Henry *et al.* (181, 182) studied the diuresis stimulated by distention of the left atrium, and presented evidence for this being a reflex response initiated by stretch receptors in the left atrium.

Effects of various adrenal steroids [Emele & Bonnycastle (183)], of the hypothyroid state [Hirvonen & Lybeck (184)], and of triiodothyronine and its azo derivatives [Hirvonen & Lybeck (185)] have been investigated in isolated preparations of mammalian heart muscle. Selye & Bois (186) reported a marked intensification by thyroxin of the corticoid-induced hyaline necrosis of heart and kidney.

EFFECTS OF DRUGS AND OTHER CHEMICAL AGENTS

Regan *et al.* (187) measured the arteriovenous differences of K^+ and Na^+ across the hearts of normal anesthetized dogs treated with acetyl strophanthidin. Administration of the drug was followed by an increase in K^+ and a decrease in Na^+ content of coronary sinus blood, and a decline in stroke work. Conn (188) studied the effects of digitoxin and anoxia on the transfer and distribution of K^+ in the heart of the intact dog, with the aid of K^{42} . The results were consistent with a decrease in the rate of K^+ transfer into the cell, in both cases. Gertler *et al.* (189) found an increase in Na^+ but no change in the K^+ content of the hearts of digitoxin-treated rabbits. Treatment with quinidine was associated with elevated K^+ and depressed Na^+ levels. Gonlubol *et al.* (190) measured Na^+ and K^+ arteriovenous differences across the normal and failing human heart, and could find no evidence for a digitalis effect on the Na^+ or K^+ balance in this organ. Reiter (191) studied the effect of strophanthin, and frequency of stimulation, on the Na^+ and K^+ content of isolated strips of rat ventricle, and Hazard *et al.* (192) investigated the effect of K^+ on digitalis toxicity. The interrelations between digitalis and K^+ were the subject of a review by Löwn (193), who suggested that the susceptibility of patients in advanced cardiac failure to digitalis intoxication may be due to the inhibitory effect of the drug on the transport of K^+ into myocardial cells already depleted of K^+ as a result perhaps, of therapy, anoxia, or both.

Johnson (194) observed an inhibition of net transport of Na^+ out and K^+ into the cells of the isolated frog sartorius muscle, by ouabain, and strophanthidin. Dihydrostrophanthidin, containing a saturated lactone ring, was inactive. Solomon *et al.* (195) studied K^+ transport in human erythrocytes with the aid of K^{a} , and found the influx inhibited by various cardiac glycosides. Fluorohydrocortisone and aldosterone were inactive, while 17-hydroxycorticosterone showed a small digitalis-like effect. It was concluded that the characteristic lactone ring is essential for activity. Tanz *et al.* (196) noted a positive inotropic effect and an increased Na^+ uptake induced by 9- α -fluorohydrocortisone in the isolated cat papillary muscle. Hyman *et al.* (197) injected digoxin directly into the renal artery of normal dogs and observed an increased excretion of Na^+ and water.

Blain *et al.* (198) administered lanatoside C to patients with various types of heart disease. Myocardial R.Q. and extraction of a fairly complete inventory of substrates showed small and inconsistent changes. Bogatzki & Staub (199) found creatinephosphate, ATP, and ADP diminished in the dilated hearts of rats poisoned with quinidine, but within normal limits in poisoned animals treated with a cardiac glycoside. The effect of digoxin on levels of cardiac glycogen in the rat was observed to vary with the age of the animals and the dose of the drug [Read & Kelsey (200)]. Wedd & Blair (201) compared the activity of lanatoside C and its aglycone, digoxigenin, on the turtle heart. The absence of the sugar moiety resulted in a less durable effect, as well as qualitative differences in activity. Brown & Wright (202) reported the isolation by paper chromatography of cardioactive metabolites recovered from the urine of rats following the administration of various digitalis glycosides. Waser (203) studied the binding of lanatoside A and K-strophanthoside by actomyosin, using an ultrafiltration technique. The drugs appeared to be firmly bound. Okita *et al.* (204) administered C^{14} -labeled digitoxin to pregnant women and studied the distribution of the drug in the fetus removed several hours later by therapeutic abortion. Penetration of the placental barrier occurred. The drug and its metabolites were found in highest concentration in fetal heart and kidney. Titus *et al.* (205) isolated a substance from mammalian tissues that exhibited a digitalis-like effect on the frog heart, and identified it as palmitoyl lysolecithin. The significance of this interesting observation awaits elucidation. The molecular complex formed by lysolecithin and cholesterol suggests an avenue for speculation.

Purpura & Grundfest (206) described a reversible blockade of cardiac synapses produced by succinyl choline in the cat. Nature of the inhibitory action of veratrum alkaloids (veratramine and veratrosine) on the accelerator nerves was studied by Innes *et al.* (207). Their interest stimulated by the work-increasing effect of the erythrophleum alkaloids, which are B-methyl or dimethylaminoethyl esters of complex organic acids, Uhle *et al.* (208) investigated a series of synthetic esters of dimethylaminoethanol for positive inotropic activity in the dog heart-lung preparation. The esters of several dicarboxylic acids were found to be effective. Popovich *et al.* (209) found no effect of nitroglycerin on the oxygen utilization of the cat heart and con-

cluded that its beneficial influence in angina is not the result of reversal of an epinephrine-induced increase in the myocardial oxygen requirement. Nitroglycerin was likewise without influence on the rate effect of adrenergic drugs. The sympathomimetic drugs, metaraminol and mephentermine, produced an increase in ventricular stroke work in acute experimental tamponade in dogs by increasing systolic ejection [Binion *et al.* (210)].

Studies of the effects on the heart of a number of metabolic inhibitors may be mentioned briefly. Covin & Berman (211) reported a positive inotropic response of the electrically driven rat ventricle strip to malonate. Permeability seems a likely factor in the observed result. Fawaz (212) studied fluoroacetate poisoning in the dog heart-lung preparation. Mechanical failure occurred in the presence of citrate accumulation, reduced myocardial creatine phosphate, and little change in ATP. The possibility that failure was due to binding of Ca^{++} by citrate was considered unlikely because of the absence of ECG findings consistent with this thesis. The role of ATP as the source of energy for contraction was questioned in view of the mechanical failure in the presence of ATP values in the normal range. It is possible that alterations in the intracellular ionic environment may reduce the contractile response in the presence of a normal supply of ATP. The mechanism of acetamide protection against fluoroacetate poisoning was investigated in heart and other tissues of the rat and guinea pig by Gitter (213). Evidence for a fluoropyruvate-induced block at the cytochrome C-reductase step in a particulate preparation from pig heart was presented by Avi-Dor & Mager (214). Takahashi *et al.* (215) observed inhibition of contractility and changes in the electrogram in the isolated toad heart treated with various sulphhydryl-binding reagents. The changes were reversed by cysteine.

PACEMAKER, ARRHYTHMIAS, AND ANTIFIBRILLATORY AGENTS

Blinks (216) observed an increase in rate associated with an elevation in right atrial pressure in a variety of isolated mammalian heart preparations. Local anesthetics had an inconsistent effect on the response. It was suggested that the rate effect is due to mechanical tension acting on the pacemaker tissue. Arora & Das (217) described the production of a maintained atrio-ventricular nodal rhythm in anesthetized dogs, involving surgical destruction of the sinoatrial node. Roberts *et al.* (218) were able to activate a ventricular pacemaker in anesthetized cats and dogs by infusing a sympathomimetic amine while the sinoatrial mode was depressed by vagal stimulation.

Paul (219) studied the bradycardia that accompanies uterine contractions in the fetal lamb. Bradycardia could be induced by a decrease in oxygen saturation of the fetal blood, a change found to be associated with uterine contraction. Nahas (220) ascribed the bradycardia seen during short periods of apnea in curarized dogs to respiratory acidosis. Reflex tachycardia (221) and bradycardia (222, 223) have been studied as conditioned responses.

Hutcheon (150) reported that norepinephrine appeared to be more effective

tive than epinephrine in eliciting ventricular ectopic rhythms in the anesthetized cat (as well as automatic contractile activity in the isolated cat papillary muscle). The adrenergic blocking agent, 2-benzyl-2-imidazoline (tolazoline) produced cardiac arrhythmias in dogs anesthetized with cyclopropane, but not with pentobarbital [Lum & Nickerson (224)]. The mechanism of induction of ventricular fibrillation by procaine in the presence of epinephrine was studied in the saline-perfused rabbit heart by Grumbach (225). Leveque (166) found the incidence of atrial fibrillation following intravenous infusion of acetylcholine in dogs was greatly enhanced if the animals were first rendered hyperthyroid. Various forms of electrical alternans were observed in single ventricular fibers of the frog heart following treatment with thyroxine and acute anoxia [Kleinfeld *et al.* (226)]. Lanari *et al.* (227), on the basis of recordings with direct cardiac leads in anesthetized dogs, concluded that the aconitine-induced atrial flutter is due to a single rapidly-firing ectopic focus, while electrically-induced flutter has a circus movement mechanism. Deranleau *et al.* (228) were impressed by the similarity between the arrhythmias produced in the isolated rat heart by chlortetracycline and versene. Temporary reversal by Mn^{++} suggested chelation of the metal co-factor of cholinesterase as the underlying disturbance. Abildskov *et al.* (229) analyzed the spread of normal nodal and ectopic excitation on models prepared from casts of human atria.

Pedersen *et al.* (230) induced ventricular fibrillation in anesthetized dogs and found drastic changes in myocardial substrate extraction as determined by analysis of blood drawn by suction from the coronary sinus. The oxygen consumption of the dog heart isolated by the Langendorff method was found to be elevated during electrically-induced ventricular fibrillation [Jardetzky *et al.* (231)].

The antiarrhythmic activity of various alkaloids of *Rauwolfia* [Arora & Madan (232)], local anesthetic agents [Harris *et al.* (233), Moll & Wallner (234), and Carden & Steinhaus (235)], and antimalarials [Arora *et al.* (236 to 243)] have been examined by a wide range of techniques. Mephentermine ($C_6H_5 \cdot CH_2 \cdot C(CH_3)_2 \cdot NHCH_3$), which antagonized the fibrillatory action of epinephrine in cyclopropane anesthesia, decreased conduction time in the dog ventricle [Stewart *et al.* (244)]. The higher resistance to epinephrine-induced arrhythmias exhibited by female rats and guinea pigs could be conferred on males by the administration of estradiol benzoate [Louwerens & Smelik (245)].

ION EFFECTS

Schreiber (246) studied cation exchange in the isolated working frog heart with the aid of K^{42} and Na^{24} . He found evidence for slowly and rapidly exchanging fractions of intracellular K^+ . The former was sensitive to external K^+ concentration, work level, and contractile failure. Entrance of K^+ into this fraction was inhibited by ouabain, which also slowed the loss of intracellular Na^+ . Other studies on ionic changes associated with the administration

of cardiac glycosides are considered in the section of this review dealing with drug effects (187 to 195).

Neidergerke (247) compared the effects of K^+ and Ca^{++} with the staircase phenomenon in isolated strips of frog ventricle. Increase in contraction amplitude without change in action potential, as in the staircase, could be achieved by increasing the Ca^{++} concentration, or somewhat less effectively, by decreasing the K^+ . The time course of these effects was considered too slow to be accounted for by diffusion through the extracellular space and too rapid to involve equilibrium with intracellular electrolyte. It was suggested that the strength of contraction is controlled by the Ca^{++} concentration in a superficial region of the cell, and that the staircase phenomenon may be due to a change therein. Penna & Garb (21) found that lowered Ca^{++} did not decrease the augmentation following a single interpolated beat in the isolated cat papillary muscle, and that elevated K^+ produced only a slight decrease. Niedergerke (248) studied the effect of Ca^{++} on contracture produced in frog ventricle strips by the application of KCl solution. Ca^{++} enhanced tension development without affecting the amplitude of the action potential, suggesting that it acts directly on the contractile mechanism or at a point of coupling between electrical and mechanical events. Effects of the chlorides of the usual mono- and divalent cations on the isolated rabbit atrium [Hirvonen (249)] and the larval and adult *Anopheles* heart [Jones (250)] were studied. Hawkins & Smith (251) found Na^+ more effective than K^+ or Mg^{++} in relaxing the ATP-induced contraction in the glycerol-extracted perfused frog heart.

Grumbach (252) studied the ventricular tachycardia and fibrillation induced by the injection of potassium chloride following epinephrine in the isolated rabbit heart. It appeared to act by potentiating the arrhythmic effects of epinephrine through production of atrioventricular block and delay in recovery of excitability. Burn *et al.* (253) found the atrial fibrillation induced in the dog heart-lung by infusion of acetylcholine and electrical stimulation could be reverted to a normal rhythm by administering KCl. An effect of acetylcholine on permeability of the cell to K^+ was advanced in explanation of this finding.

Reports dealing with K^+ in relation to the heart in hypothermia [Mavor *et al.* (254, 255)] and with the concentration of various ions in the normal [Mulder *et al.* (132)] and failing dog heart [Benson *et al.* (141)] may be cited. Robertson & Peyser (256) found the volume of distribution of sucrose in the myocardium was consistently smaller than that for Na^+ and Cl^- .

Several papers concerned with the effects of ions on electrical events are cited in the section of this review dealing with electrophysiology of the heart (269 to 271).

ELECTROPHYSIOLOGY

Hutter & Trautwein (172) recorded action potentials from sinus venosus and atrial fibers of the spontaneously beating frog heart using intracellular

microelectrodes. A slow diastolic depolarization (pacemaker potential) characterized the sinus fibers. This potential was suppressed by vagal stimulation, which failed to alter the diastolic membrane potential of atrial fibers. In both sinus and atrium, vagal stimulation accelerated repolarization of the action potential and reduced its amplitude. Sympathetic stimulation increased the rate of rise of the pacemaker potential.

Webb (257) applied the microelectrode technique to the electrically driven atrium of the rat. In general, a rapid rate of repolarization was associated with a high resting and action potential. The appreciable variation found in a large number of trials (1866) was considered a reflection of actual differences in the cell population. In addition to a decrease in the rate of conduction, acetylcholine produced a marked shortening of duration of the action potential by speeding repolarization [Webb & Hollander (173)]. Epinephrine gave opposite effects, both electrical and mechanical. Similar studies in the sino-atrial node and atrial fibers of the rabbit heart were reported by Cervoni *et al.* (258) and West *et al.* (174). Sino-atrial cells showed action potentials of longer duration than atrial cells, which were able to follow an electrical pacemaker at higher rates. A greater tension response was associated with prolonged repolarization time in the atrium. In the sinoatrial node the slowing produced by acetylcholine was associated with a reduction in prepotential slope and magnitude of the action potential. The epinephrine rate effect was characterized by an increase in the prepotential slope without change in the magnitude of the action potential. After a period of quiescence in the frog ventricle, Niedergerke (247) observed full-sized action potentials associated with the first small contractions. In the isolated rabbit atrium cooled to 14° to 20°C., mechanical and electrical activity ceased except for small rhythmic nonpropagated potentials in the region of the pacemaker [Marshall & Vaughan Williams (164)].

Van Dam *et al.* (259) confirmed the classical findings with respect to the contour of the excitability curve of the dog's left ventricle during the relative refractory period. Mendez *et al.* (260) found that, in general, the shortening of the refractory period with increasing heart rate did not develop as a cumulative effect, but was a function of the length of the immediately preceding cycle. Dawes & Vane (261) studied the effect of variations in conditioning and test stimuli on the refractory period in the isolated atria of mammalian hearts. Hegnauer & Covino (262) reinvestigated ventricular thresholds in the hypothermic dog, and in contrast to their own prior findings observed only slight deviations from normal. The discrepancy was assigned to technical inadequacy of the earlier work.

Fänge *et al.* (163) investigated the resting and action potential of cultured embryonic chick heart using intracellular microelectrodes. Findings were similar to those in the intact embryonic heart. Lazzarini & Bellville (263) described a method for recording the electrocardiogram of the chick embryo within the shell. Sano *et al.* (264) studied the action potentials of single

atrial and ventricular fibers of the tortoise heart. The electrogram of the cicada heart was investigated by Irisawa *et al.* (265) using surface and intracellular microelectrodes. Evidence was obtained indicating the presence of two pacemakers.

Moe *et al.* (266) studied the propagation of early premature responses between the atrium and ventricle of the dog heart. The results were interpreted as consistent with the presence of a dual atrioventricular transmission system, in which the components are distinguished by differences in refractory period and conduction interval. Scher & Young (267) presented a three-dimensional analysis of the pathway of depolarization in the dog ventricle. In a unique isolated rabbit atrium preparation, Rijlant & Reuse-Blom (268) were able to demonstrate impulse conduction through a segment of tissue in which the mechanical response was completely inhibited.

Hoffman & Suckling (269) studied the effect of changes in the concentration of Ca^{++} , K^{+} , and Mg^{++} on the resting and action potentials of single fibers of atrium, ventricle, and conducting tissue of the dog heart. It was suggested that the normal differences in the action potentials of the three tissues may be due to differences in sensitivity to extracellular Ca^{++} . Weidman (270) studied the effect of brief injections of K^{+} into the coronary circulation of the turtle heart. Abruptly raising extracellular K^{+} in this manner during diastole produced transient depolarization; early in systole a shortening of the action potential resulted. Excitability, conduction time, and refractory period in the isolated frog heart were investigated as a function of changing K^{+} concentration [Benthe (271)].

Studies on the effect of mephentermine on ventricular conduction time [Stewart *et al.* (244)], the relation between membrane potential and contractility in the presence of anoxia, substrate depletion, or dinitrophenol [Webb & Hollander (36)], the effect of quinidine, procaine amide, and pyrillamine on membrane potentials of ventricular fibers [Johnson (272)], and the effect of various local anesthetic agents on the refractory period of isolated rabbit atrium [Luduena *et al.* (273)] have been published. Weidmann (274) reviewed the subject of the electrophysiology of the heart muscle fiber.

The voluminous literature on electro- and vectorcardiography, which has been given appropriate recognition in previous years, has not been covered in the present review because of limitations of space.

ANATOMY

Augustinsson *et al.* (176) made a comparative study of autonomic innervation and histological structure in cyclostome hearts (lamprey and hagfish). Structural features of the crocodile heart which effect the separation of aerated and nonaerated blood were pointed out by White (275). Hibbs (276) applied electronmicroscopy to a study of developing cardiac muscle in the chick embryo. At about 30 to 36 hr. incubation the first thin Z bands appeared, and myofilaments which were present previously became oriented

to form bundles. The first contractions also occurred at this time, approximately twelve hours before the appearance of A substance and other fine structure.

Fox & Goss (277) produced a high incidence of congenital cardiovascular lesions in rats, with interventricular septal defect a prominent finding, by the administration of trypan blue to pregnant females. In hearts of rats four to seven days of age examined from 24 hr. to 14 days after trauma to the myocardium, Robledo (278) found histological evidence for regeneration of myocardial fibers both by mitotic and amitotic division.

Irisawa & Irisawa (279) mapped the location of the pacemaker in the toads heart by the application of a small thermode to provide local heating. A highly thermosensitive area (as judged by the rate response) could be defined which corresponded to Remak's ganglion.

Oppenheimer *et al.* (280) reported the use of carbon dioxide as a contrast medium for visualizing intracardiac structures by cinefluorography.

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RUSSIAN PHYSIOLOGY (CARDIOVASCULAR ASPECTS)^{1,2}

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INTRODUCTION

Reading the cardiovascular literature published in U.S.S.R. from 1952 to 1956, one is impressed with the prominence of references to Pavlov. Among the 500 odd papers this reviewer read in preparation of this article, there were few that did not refer to Pavlov in one or another context, some references going back as far as 1882. In Chernogorov's (1) monograph on angina pectoris, Pavlov is quoted more frequently than any cardiologist, and even in Dekhtar's (2) textbook on electrocardiography Pavlov is frequently quoted although he never was active in this field. Over 400 papers on Pavlov published from 1949 to 1952 and 335 papers from March 1953 to April 1954 are listed in two editorials of the *Fiziologicheskii Zhurnal S.S.S.R.* (3, 4). This bibliography includes the most prominent authors not only in physiology and neurology, but also in surgery, otolaryngology, obstetrics, etc. Pavlov's concepts were introduced as guiding principle for work in all fields of medicine, and physiology in U.S.S.R. is now in general called "Pavlovian Physiology" (5). The overwhelming influence of one great physiologist on research work in the wide field of biology and medicine in the U.S.S.R.—in recent years spreading to Poland, Czechoslovakia and East Germany—is without precedence in the history of medicine.

Pavlov's influence has led to emphasis, perhaps to some overemphasis on the role of the nervous system in cardiovascular physiology and pathology. It has stimulated research in this area on a large scale, unparalleled elsewhere, but it seems to have limited research and development of new techniques in other areas. The differences in approach, resulting from Pavlov's influence, may be illustrated by comparison of the discussion of an important finding made independently and nearly simultaneously in the United States by Burch *et al.* (6) and in the U.S.S.R. by Popov (7): a large and wide inverted T waves in cerebral hemorrhage in the absence of cardiac disease, suggestive, in the first days, of myocardial infarction. However, recovery was much faster than in typical infarct. Burch *et al.* stated that the mechanism is unknown, but suggested electrolyte disturbance as the most likely

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cause. Popov goes as far as to suggest that the general assumption of a relationship between morphological changes in the infarcted myocardium and ECG changes might not be correct. While general acceptance of such an extreme view appears to be unlikely even in the U.S.S.R. the emphasis on central nervous system effects agrees well with the general approach and reasoning. In fact, the phenomenon is quite in the line of expectation in U.S.S.R. and somewhat outside the line of expectation in the United States.

There are also numerous references to the work of Vedenskii and Ukhtomski. Their influence on present medicophysiological research is second only to Pavlov [Chernogorov (8)]. Of particular interest is Vedenskii's concept of "lability" and of "parabiosis." Lability is an index of excitability, related to the refractory period. It is the maximum number of impulses which can be generated or conducted per unit of time; therefore, increased lability means increased excitability. "Parabiosis" is the designation for the complex changes of excitability which take place in the process of inhibition. It can be produced by a variety of injuries and is interpreted as a locally frozen nonpropagated state of excitation, i.e., an active process, which however exerts some effect on the surrounding tissue (perielectrotone). One characteristic feature of this state is the changing relationship between effects of high and low frequency stimulation, and of strong and weak stimuli; ultimately, a weak stimulus produces a greater response than a strong stimulus. This "paradoxical phase" is also an important feature in abnormal conditioned reflexes.

In view of space limitation, an arbitrary selection of topics and publications was necessary. The bulk of the large material on arterial hypertension and coronary artery disease will be reviewed elsewhere.

HEART

ELECTROCARDIOGRAM

Excitability and conductivity.—Vedenskii's phenomenon of "optimum" (maximum) and "pessimum" (minimum) contraction, obtained by variation of strength and frequency of stimulation, was reproduced by Priima (9) in the electrically stimulated frog ventricle, arrested by means of a Stannius ligature. The optimum (maximum contraction) occurred at a stimulation rate corresponding to the natural rhythm.

The problem of atrioventricular (A-V) conduction has been studied with particular attention. In meticulous experiments, Daue & Shidlovskii (10) found in the isolated frog heart by means of successive ablation that of the three atrial muscular layers, only the trabecular bundles are capable of A-V conduction. The critical number of trabecular bundles (diameter of 30 to 40 μ , composed of fibers 3.5 to 4 μ in diameter) for unimpaired A-V conduction is about fifteen; first degree or partial A-V block is produced by reduction of the number of bundles below 15, and complete A-V block by reduction below seven to five (11).

The normal unidirectional A-V conduction depends on the resting po-

tential gradient between atrium and ventricle and not on the atrial refractory period. Its elimination by cooling of the atrium abolishes this property and retrograde conduction takes place [Ugel'nov & Popova (12)]. The P-R interval is lengthened by D.C. current of 1.5 to 2 mA with the cathode on the atrium and the anode on the ventricle of the frog's heart. Reversal of the electrodes shortens the P-R interval (13) which, according to the authors, may have application to the Wolff-Parkinson-White (WPW) syndrome.

Lirman (14) produced in dogs under pentobarbital anesthesia progressive shortening of the P-R interval and lengthening of the QRS interval, resulting in typical WPW complexes by stimulation of left (but not right) cardiac nerves, at frequencies from 50 to 100 c.p.s. Normal conduction was restored 30 to 60 sec. after the end of stimulation. Since pentobarbital anesthesia blocks the vagus nerve it is assumed that production of the WPW syndrome is a sympathetic effect, disturbing the equilibrium of right and left bundle branch conductivity. This assumption was supported by symptoms of sympathicotonia in all 27 patients with WPW studied by the author, but is not easy to reconcile with restoration of normal conduction after atropine. The possibility of extracardiac nervous influence in the production of the WPW syndrome in some patients, of course, does not preclude its production by localized ischemia due to arteriosclerotic changes in two small descending branches from the right coronary artery, supplying the atrioventricular node and the left bundle branch [Sigal (15)]. Atropine produces normal conduction only in the first phase of development by improvement of the blood supply through reduction of vagal tone. In later phases, the WPW syndrome becomes stable, and further progressive development may produce nodal rhythm and tachycardia, sometimes with transition to ventricular tachycardia. The various causes (nervous effects and organic involvement) may help to explain the differences in clinical significance of the WPW syndrome for different patients. Slepneva & Golovko (16) found WPW syndrome in a child of six years with situs inversus, an unusual combination.

Chernogorov (8) produced partial A-V block in dogs by local application of 12 to 20 per cent chloralhydrate. Placing the anode of a D.C. current on the A-V node restored normal A-V conduction, while catelectrotonus produced complete A-V block. In first degree or partial A-V block, weak atrial stimuli were better conducted than strong stimuli, similar to Vedenskii's "parabiotic" condition in a damaged peripheral nerve. Retrograde impulses from the ventricles frequently increased the A-V block.

Acute experimental damage of the A-V node often produced complete atrial arrest, and sometimes even ventricular standstill, suggestive of a closer functional relationship between A-V node, atria, and ventricles than mere conduction. This is substantiated by studies of ventricular and atrial excitability in experimental A-V block. The chronaxie of the ventricle and of the sinus node nearly always increased immediately after application of 25 per cent chloralhydrate to the A-V node in the isolated heart of the dog perfused *in situ*, but there was no correlation between the degree of A-V

block and the increase of chronaxie. Ischemic, thermal, and chemical ventricular lesions [Sidorenko quoted from Chernogorov (8)] of the heart in dogs shortened and later lengthened the chronaxie in the damaged area, and produced opposite changes 30 to 40 mm. distant. These remote effects of experimental A-V block and ventricular damage agree well with Vedenskii's "perielectrotone."

A-V block could be abolished in dogs by low concentration (1:10,000,000) of epinephrine, while stronger concentrations had no effect, or even increased the degree of A-V block. In a patient rectal infusion of 50 cc. of epinephrine (1:200,000) within 20 min. converted a complete A-V block to partial 2:1 A-V block, and later to first degree A-V block. The improvement in this and other patients lasted for one to two days. Subcutaneous injection of 1 cc. 1:1000 epinephrine or 1:100 atropine was without effect (8). Transient improvement of A-V block in the isolated heart of the dog occurred after physostigmine (1:1,000,000), atropine, and isotonic CaCl_2 , while 0.79 per cent KCl usually increased the degree of block. However, when KCl is applied shortly after CaCl_2 , it may improve A-V conduction, and Ca applied shortly after K, may delay A-V conduction.

Chernogorov's view of the "parabiotic" nature of the A-V block is shared by Matusova (17), but opposed by Dolgoplosk & Shadur (18) in a thorough discussion of the problem. One of the objections is the difficulty of conceiving that an active local excitatory process, which is the basis of Vedenskii's parabiotic state, can be maintained for years in patients with chronic A-V block.

Monophasic injury currents.—Investigations of Udelnov and his associates have led to knowledge about a new aspect of monophasic injury currents. Placing a piece of necrotic tissue (muscle, liver, lung, etc.) on the intact frog, rat or cat ventricle at the site of the electrode instantaneously produced monophasic currents, which could not be differentiated from true injury currents obtained by trauma at the same location (19, 20). Using the standard leads instead of direct bipolar leads, patterns simulating acute anterior, posterior or lateral infarction were obtained depending on the location of the placement of necrotic tissue. The effect was quickly reversible after removal, but a transient T inversion of the coronary type occurred for several minutes before the ECG became normal. Tissue applied immediately after removal from its original site had no effect; the first effect on the ST segment appeared 20 to 30 min. after removal, and the full effect was obtained after keeping the tissue *in vitro* for one and one half to two hours. Tissue in the process of necrosis *in situ* after experimental ischemia, had the same effect as isolated tissue. The magnitude of the monophasic potential was proportional to the size of the piece of necrotic tissue. Functional impairment of ventricular contractility in the region of the piece of necrotic material was demonstrated by motion pictures (21). Monophasic currents were obtained from a single atrial trabecula in the frog heart, using the same technique (22). The authors suggested that this phenomenon may be involved in the

pattern of acute myocardial infarction and in other types of ST deviations. Placement of two necrotic pieces, of approximately the same size, at anatomically opposite points in line of the lead axis cancelled the monophasic deviation (23, 24). No control experiments with local potassium application were reported.

Necrotic tissue placed on or close to the A-V node, produced A-V block (from first degree to complete) (25), and sometimes the WPW pattern (26), which is of considerable interest for the mechanism of transient A-V block in acute myocardial infarction.

Vagal stimulation moved the baseline in the direction of the monophasic action currents, thus decreasing the amplitude of their deflection (27) and similar effects could be obtained with polarization through D.C. current: the anode, placed on the injured frog ventricle, decreased the ST elevation, and the cathode decreased the T inversion (28).

Arrhythmias.—Comparatively few studies on atrial arrhythmias have been reported. Osol (29) found the range of the atrial rate in flutter (from 212 to 460 per min.) and in atrial fibrillation (from 294 to 530 per min.) overlapping and there were also no significant differences in the amplitude which confirms larger material probably not known to the author.

However, atrial fibrillation waves were significantly higher in 31 patients with rheumatic heart disease (mean, 0.212 ± 0.01 mV), than in 20 patients with arteriosclerotic heart disease (mean, 0.068 ± 0.01 mV) (30). Shestakov (31) studied the relation of atrial fibrillation to the underlying disease, various electrocardiographic findings (extrasystoles, QRS interval and axis, ST and T changes, bundle branch block), arterial pressure and constitutional factors (age, sex) in a large group of patients. Atrial fibrillation was found only in 6.7 per cent of patients with myocardial infarction in Shestakov's study. The incidence of arrhythmias of any type, on the other hand, was 48 per cent in 335 patients with myocardial infarction and increased with the size of the infarct [Teodori (32)].

Electrical field.—Electrical field distribution was studied by Shidlovskii & Markovskaia (33) in the isolated frog heart placed in the center of moist filter paper. Immersion of the ventricle into the inactive larger atrium (thickness, 0.5 mm.) decreased the amplitude of the ventricular complexes by 45 per cent, and into the inactive ventricle of a larger frog (thickness 3 to 4 mm.), by 80 per cent. These experiments were performed in order to demonstrate that the recording of electrical currents in the inner layers is minimized by the outer layers. Increase of right atrial pressure moved the P-axis of the isolated frog heart (34), the isolated and the *in situ* cat heart (35) to the right, and increase of left auricular pressure to the left. It was not possible to differentiate between pressure and dilatation effects. Emptying of the ventricular cavity, or replacement of Ringer solution by vaseline or distilled water increased the QRS amplitude of the isolated frog heart (placed in the center of a circle of moist paper) by two to three times. Dilatation of the ventricle by overfilling with Ringer solution decreased the amplitude by 50

per cent [Shidlovskii & Iastrebtsova (36)], which might have some application to the low voltage often found in patients with cardiac decompensation.

Heart metabolism.—Adenosintriphosphate (ATP), in a concentration of 10^{-4} , increased the R wave of the isolated frog heart by 50 to 75 per cent after five minutes perfusion; an increase of the T wave, Q-T prolongation and shortening of the P-R interval occurred later, independently of the QRS changes. It was concluded that ATP affects depolarization primarily [Kelareva (37), Feldman & Kiandjuntseva (38)]. Perfusion of the frog heart with thiamine in concentration of 10^{-2} to 10^{-3} produced A-V block, and in concentrations from 10^{-4} to 10^{-8} increased the QRS, and frequently also the T amplitudes [Iastrebtsova (39)].

In 15 rabbits with ventricular hypertrophy produced by experimental hypertension, the glycogen and phosphocreatine content in the heart dropped 37 and 59 per cent, respectively, as compared with 15 controls. Inorganic phosphate increased slightly (+10 per cent); there was no significant change of ATP or dry substance. Left ventricular strain pattern developed only in 7 out of 15 animals. In all animals with abnormal ECG the decrease of glycogen and phosphocreatine was pronounced, but it also occurred in animals without abnormal ECG changes. Biochemical changes appear to precede the ECG changes [Raiskina (40)].

In dogs with experimental pancreatic diabetes the initially positive T wave became inverted one to two weeks after the operation, but previous thyroidectomy delayed the T inversion by several weeks. There was no correlation between T wave changes and blood glucose level, and the QRS complex was not affected [Popov (41)].

Cadmium chloride increased the P-R interval of the isolated frog heart and later produced 1:2 A-V block and inversion of the T wave. Cysteine restored the electrocardiogram to normal. Urea increased the amplitudes of the R wave and T wave, but later the T wave became diphasic. Since cadmium chloride combines with the SH group and urea and cysteine supplies free SH groups, it was suggested that the SH group is of importance for the genesis of the electrocardiogram [Logunova & Kipershlak (42)].

Effects of central and autonomic nervous system; reflexes.—Popov's (7) observation of large inverted T waves in two patients with cerebral hemorrhage and normal heart was already mentioned in the introduction. In one patient of 26 years with normal ECG, A-V block developed gradually from first degree to complete block after head trauma (18). In animal experiments, decerebration produced sinus bradycardia, arrhythmia, and prolongation of the P-R interval [Britvan & Kydin (43)]. Stimulation of the midbrain region in dogs produced A-V and intraventricular block [Livshits (44)] and transcranial electrical stimulation, similar to shock therapy in mental patients, also produced ECG changes [Azhibae (45)]. In experimental neurosis produced by conflicting conditioned stimuli in lower monkeys, an abnormal T inversion developed gradually increasing through the experimental period of two years [Kokaia (46), Magakian & Miminoshvili

(47, 48)]. In the initial phase, nitroglycerine normalized the ECG, but later the changes became permanent. In most of the animals, arterial hypertension developed at the same time, but there was no correlation between hypertension and ECG changes. The T-inversion was more prominent than the ST depression, which was often absent; the pattern, therefore, was more typical for coronary insufficiency than for left ventricular strain. In one animal, the typical pattern and evolution of posterior wall infarct appeared, which was confirmed by autopsy. Compared to the control animals, all experimental conditions were identical, including the diet. This series is one of the most important documentations of the potential involvement of nervous factors in the pathogenesis of coronary insufficiency.

In another series on twenty lower monkeys with experimental neurosis, pressure on the eyeball increased the T wave and produced partial A-V block in some animals [Djalagonia (49)]. In three patients with emotional upset and one patient with postconcussion syndrome, a prominent Q wave and T inversion in leads II and III simulated the pattern of a posterior wall infarct in the absence of clinical evidence for infarction [Karamyshev & Koreisha (50)]. T and ST changes in emotional upset are well known, but QRS changes are unusual.

Infusion of 200 to 400 cc. into the urinary bladder through a catheter produced prolongation of the P-R interval and T changes together with an increase of the arterial pressure. These changes were more pronounced in 11 patients with pyelocystitis, and 23 patients with arteriosclerotic heart disease than in 13 normal subjects. Local novocaine application abolished the response [Ganelina & Tsbibel (51)].

Stimulation of an isolated intestinal loop perfused *in situ* with 10 to 50 γ acetylcholine did not change the ECG of normal cats essentially, but produced ventricular extrasystoles and T inversion in cats with experimental myocarditis [Ganelina (52)]. While reflex changes of the ECG are documented in the international literature, the possibility of myocardial infarction developing on this basis, as claimed by Prikazchikov (53), is somewhat doubtful; the ECG's of his two patients were not entirely typical for acute infarction.

Electrical stimulation of the vagal center in the medulla oblongata produced, in addition to A-V block and bradycardia, gigantic T waves and large Ta waves in frogs, and also occasionally increased the QRS complex [Shidlovskii & Kiandjuntseva (54)]. The results may have some bearing in the large T waves observed in cerebral hemorrhage (7).

Nodal rhythm, interference dissociation, decrease of the P-R interval, increase of the QRS interval, T changes (which were not uniform), and, in one animal, transient ventricular fibrillation developed during stimulation of one ("Pavlov's") branch of the left cardiac nerves in dogs [Raiskina (55)].

Chemical stimulation of the vagal centers by means of sodium chloride crystals placed on the dorsal medulla oblongata in frogs [Udelnov (56)]

produced changes of the monophasic electrogram of the injured ventricle, which were independent of the vagal bradycardia and reversible after removal of the crystals. Similar changes were found by the same author in other types of reflex cardiac inhibition (57). In this connection, the development of myocardial degeneration and ECG changes similar to those in coronary insufficiency in experimental neuritis of the vagus nerve in rabbits is of interest [Chernukh & Iushtshenko (58)].

In the exploration of nervous effects on the ECG conditioned reflexes have been used by various investigators. The variety of reactions which could be utilized is of interest. Bradycardia, prolongation of the P-R and Q-T intervals, and transient increase of the T wave by about three times the original amplitude, appearing after intravenous "pituitrin" injection in dogs, could be reproduced by a conditioned acoustic signal applied 30 sec. before pituitrin injection [Teplov (59)]. The conditioned response obtained after 80 to 120 repetitions was in no way different from the direct "pituitrin" effect. Once established, the conditioned reflex was very stable; it disappeared only after 150 to 160 repetitions without reinforcement. Chernogorov (8) obtained conditioned nitroglycerine effects (ST and T changes) after 100 repetitions. Electrical skin stimulation in dogs shortened the R-R and P-R interval, and increased the P wave, R wave, and T wave. These changes could be produced by a conditioned acoustic stimulus [Toptshiyeva (60)]. In several subjects with ventricular extrasystoles after exercise or during pressure on the eyeballs, verbal signals associated with the procedure reproduced the extrasystoles. This conditioned reflex disappeared when the verbal signals were repeated without reinforcement [Peimer (61)].

Of great interest is interaction between EEG and ECG; profound changes of the EEG occurred during angina attacks, together with the typical ST depression in the ECG [Vovsi (62)] and Subbotin *et al.* (63) found in patients with paroxysmal atrial tachycardia an abnormal EEG pattern very similar to epilepsy. Altykhov & Malkin (64) found in normal subjects that ECG and EEG changes occurred simultaneously during breathing of 9 per cent oxygen.

Response to stresses.—Exercise and anoxia tests used mainly for detection of latent coronary insufficiency [Soboleva (65), and Vinogradov (66)] corroborate the experience outside the U.S.S.R., but the testing procedures appear to be less uniformly standardized than in the United States.

Butchenko (67) suggested that the ECG should be taken not only after, but also during exercise; he found that in 6 out of 32 patients important changes, particularly extrasystoles, were missed in the recovery ECG. Although nitroglycerine reverses the ST depression or T inversion in spontaneous or experimental (exercise, anoxia) angina attacks, it was of little diagnostic value in patients with chronic coronary insufficiency [Tur (68), Dibner (69, 70)]. The ECG response to cooling of the arm also was diagnostically worthless [Shulutko (71)]. Dibner (69, 70) in contrast to Soboleva (65), stresses the potential value of the orthostatic test. In dogs with experi-

mental hypertension, the response to the anoxia test became abnormal before the resting ECG did [Minterev (72)].

Intravenous injection of 60 cc. Ringer solution into normal rabbits did not change the ECG significantly but after partial pneumonectomy produced greater ST depressions than 30 min. exposure to 50°C., or exercise (running 85 m. in a wheel) [Miliagin & Romanova (73)]. In 19 patients with partial pneumonectomy, oral intake of 500 cc. water produced changes similar to slight right ventricular strain, and this test was more sensitive than other stress tests, although the individual variability was large (73). Increased intrapulmonary pressure from inflation by a tracheal catheter (13 to 16 mm. Hg for 20 sec.) produced atrial and ventricular premature ventricular beats, atrial fibrillation, sinoatrial block, intraventricular block, ST depression, T inversion, or gigantic positive T waves in 10 out of 14 rabbits with experimental hypertension. These changes were more pronounced in the later phases of hypertension, but could be diminished or prevented by bilateral vagotomy. No abnormal deviations occurred in nine controls [Kvitnitskii (74)]. Shestakov (75) made the interesting observation that various intercurrent noncardiac diseases in patients with healed myocardial infarction may reproduce an earlier phase of the evolution, similar to episodes of coronary insufficiency.

Miscellaneous.—Study of 68 cases with heart trauma afforded an unusual opportunity to compare the localization of embedded foreign objects by means of x-ray and electrocardiography [standard and CR chest leads c.f. Ivanov & Soboleva (76)]. In 31 patients, the ECG was normal. In the remaining 37 cases, the ECG changes did not correlate with the size of the object, and not always with the x-ray localization. The agreement was best in posterior wall location. Sometimes, the changes simulated the pattern of myocardial infarction, but in most patients, they were comparatively small, and developed occasionally several years after the trauma. In 5 out of 12 patients the exercise tolerance test was abnormal.

Hypoxia, produced by experimental methemoglobinemia in dogs, increased the heart rate and the T wave, which was followed in the terminal phase by ST depression and T inversion. The R wave decreased continuously (43). From analysis of clinical correlates of a high precordial T wave in 230 patients, Soliterman (77) concluded that it is most likely abnormal, and probably attributable to left ventricular ischemia. The high T wave in the initial phase of methemoglobinemia seems to corroborate this interpretation, and in this connection it is of interest that heavy anerobic exercise produces very large T waves in young normal men (78). Heart standstill for three minutes during surgery (denervation in a patient with bronchial asthma) produced ST₂ depression and T₂ inversion, which recovered within 48 hr. [Sokodi-Dimitrov & Gerge (79)].

ECG changes compatible with ischemic heart disease were common in 155 patients with peripheral arteriosclerosis, even in absence of cardiac symptoms, a fact demonstrating the sensitivity of the ECG in detection of early

myocardial involvement [Lashtshevker (80)]. Similarly, the development of a left ventricular strain pattern preceded the development of anatomic left ventricular hypertrophy in 68 rabbits with experimental hypertension [Kvitnitskii (81)]. In contrast to the clinical experience with hypertensive patients, there was a correlation between the extent of ECG changes and the increase of arterial pressure, possible because of the simpler experimental situation.

Electrocardiographic changes occurred early also in arterial hypertension produced in nine monkeys by means of daily intravenous injection of "pituitrin" [Belous & Magakian (82)].

In experimental cardiosclerosis produced by cholesterol-feeding in 92 rabbits, the decrease of the QRS voltage, but not ST and T changes, paralleled the development of diffuse myocardial degeneration. ECG records often revealed histologically confirmed focal damage before development of diffuse degeneration [Kipshidze (83)].

Giliarevski *et al.* (84) found a positive correlation between changes of the Einthoven P and QRS axes in 178 patients with mitral heart disease, which, however, was not high enough for individual prediction. The report of one case with T inversion after interpolated premature ventricular beats is of interest [Sigal (85)]. The more common T inversion after the usual type of premature beats with compensatory pulse is believed to be causally related to the increased diastolic filling in the prolonged R-R interval. Since the pause is shortened after interpolated beats, another mechanism must be involved.³

Negovskii & Soboleva (86) studied the terminal ECG of dogs in acute hemorrhage. The Q-T interval became prolonged up to 0.6 to 0.8 sec., and the QRS complex splintered. After cessation of respiration and detectable peripheral pulse, either rare ventricular complexes occurred (one to two per min.), or slow (80 to 120 p.m.) ventricular fibrillation of low amplitude.

For experimental work in animals, the normal range of the most frequently used ECG items in 32 dogs compiled by Gurevich & Kvitnitskii (87) in 68 rabbits (81) and in 125 cats by Angarskaia *et al.* (88) is valuable. Sapov's (89) findings of large spontaneous intraindividual variability of the T wave in dogs in observations lasting up to 10 months are of importance for chronic experiments. Kokaia (46) determined the inter- and intraindividual normal variability in 44 lower monkeys, over a period of two years. In general, the monkey ECG is very similar to the human.

Clinical electrocardiography; summary.—The bulk of clinical electrocardiography is outside the scope of this review. Some general impressions, however, may be of interest. The most commonly used precordial leads are the CR leads, but the V leads are now in the phase of introduction (2). There are some recent studies of the diagnostic value of the aV-leads (90, 91) and of the V-chest leads (92, 93, 94). In this respect, the ECG literature in U.S.S.R. is very similar to that in the United States in the mid-forties.

³ The reviewer has several similar cases in his file.

Masliuk (95) stresses the diagnostic value of the P:QRS amplitude ratio in esophageal leads, which was increased in 91 per cent of patients with mitral stenosis, as compared to 44 per cent in the conventional limb and chest leads.

The search for lead systems with a minimum of electric distortion, which plays such a prominent role in the Western electrocardiographic literature, does not seem to have an equivalent in U.S.S.R., and the same can be said for research in spatial vectorcardiography. The only consideration of QRS loop projection on standard leads was found in a paper by Sisoiev (96) for measurement of the QRS interval. The old concept of partial right and left electrocardiograms still finds adherents [Lashtshevker (97)].

Some of the normal limits given in Dekhtar's textbook (2) would, if applied to American population, result in a very large number of false diagnoses of abnormalities. Significant differences of electrocardiographic characteristics in different populations would be of great interest, but the source of these limits was not given.

HEART MOTION

A detailed analysis of contour and volume of the ventricular cavity in the isolated heart of cats, perfused with contrast solution in defibrinated oxygen-saturated blood was made by means of x-ray kymography in the frontal and lateral planes [Glikin (98)]. The residual volume at the end of systole is one-fourth to one-third that of the diastolic volume. The spread of contrast material inside the human heart, a few seconds after injection, was recorded by means of x-ray angiocardiology [Meshalkin (99)].

X-ray kymography was used for analysis of respiratory changes of the heart and large vessels: the pulsations of the pulmonary artery increase in inspiration and decrease in expiration, with opposite changes in the aorta. The decrease of the heart volume in the Valsalva procedure occurs first in the right atrium and ventricle, and later in the left ventricle, particularly in the outflow tract, together with a marked decrease of the pulsations of aorta and pulmonary artery, and shortening of the ejection time [Tikhonov (100)]. The relationship between heart sounds and the motion of the heart as recorded with x-ray kymography simultaneously on one film was studied by Savchenkov (101). In ventricular aneurism, x-ray kymography was valuable for localization and appraisal of contractility in the surrounding parts of the myocardium [Dobashvili & Tavonius (102)]. Photoelectrokymography is represented only in one preliminary communication [Zaretskii (103)].

Parin reviewed some of the recent American literature (104), and illustrated the application of Dock's simple ballistocardiographic method in small normal and abnormal groups (105, 106, 107), with the purpose of introducing ballistocardiography in U.S.S.R.

It appears that Babskii's *et al.* "cardiohemodynamography" has to a certain degree replaced ballistocardiography in the U.S.S.R. Four sensitive tensiometric levers, arranged in a square and ballanced in a Wheatstone bridge, record the movements of the chest wall, corresponding to the shift

of the mass of blood, through imbalance of the bridge on an oscilloscope after amplification. Basically, therefore, the method records the same phenomenon as ballistocardiography, and is similar in principle to that described by Brandt *et al.* (108). Naturally, the contour of the curves recorded from different parts of the body is quite different (109). The authors identify seven phases (isometric contraction, rapid ejection, etc.), but the basis for their identification is not quite clear, no reference to simultaneous heart catheterization being given. Although the inertia of the pick-up is small, the inertia of heart and blood movements is large, and it would appear that any method involving inertia of larger masses is not ideal for analysis of time intervals, but this is the main application of the method. While the breakdown into partial intervals may be somewhat questionable, the values for the total mechanical systole (S) fit very well into the range obtained with other methods, and the close correlation with cycle length (deviation from the regression equation: $S = 0.32 \sqrt{\text{cycle length}}$ (or $0.183 \text{ cycle length} + 0.142$) was ± 0.02 for 95 per cent of the sample of 80 men) speaks for the internal consistency of the method. The mechanical systole was shorter than the electrical systole, in confirmation of current concepts; this difference was found to increase with $\sqrt{\text{cycle length}}$ (110).

Significant differences between the contours of the curves of normal subjects and of patients with valvular heart disease were found (111). In patients with mitral stenosis the intervals defined as atrial and isometric ventricular contraction were prolonged, and in aortic insufficiency the amplitude of the rapid ejection period was substantially larger.

The results were confirmed in a later series (112). The cardiodynamograms of 16 out of 37 patients with mitral stenosis became normal after surgery. The duration of isometric contraction was 0.127 ± 0.018 sec. before, and 0.105 ± 0.023 sec. after operation (113). In patients with myocardial infarction, the amplitudes of the fast ejection phase were decreased together with large beat to beat variability (111). Parallel to recent development in ballistocardiography, the movements were simultaneously recorded in different planes as basis for tridimensional vector-analysis (114). Iankevskii (115) concludes, on the basis of 500 experiments, that Babskii's cardiodynamography is still in the phase of exploration; the normal and pathologic variations are not yet sufficiently known, but the results are promising for diagnosis of early stages of cardiovascular disease.

A portable instrument for recording of movements, with sensitivity of displacement of 0.01 cc., was developed for recording of heart movements from the epigastrium. The contours of tracings in a small number of patients with valvular heart disease and coronary insufficiency were different from normal subjects [Djordjikia & Voitsekhovskii (116)].

The material obtained with Babskii's cardiodynamography cannot compare in quantity, standardization (117) or mathematical-physical analysis of background and sources of error (118) which has been developed

in ballistocardiography. It appears that any well standardized, sensitive method of recording heart movements will show significant differences between normal subjects and patients with distinct cardiac pathology.

The frequency distribution of heart sounds was studied with 27 filters over a range from 36 to 18,000 c.p.s. in normal subjects and a small number of cardiac patients [Oleinik (118)].

Heart catheterization seems to be used on a comparatively small scale, but Meshalkin's (119) recent monograph is quite comprehensive and up-to-date. Keshisheva (120) described a photometric manometer for intracardial pressure recording.

CARDIAC DECOMPENSATION

During acute cardiac decompensation produced in rabbits by means of experimental aortic stenosis, the Q-T interval increased, T_1 became diphasic or negative, and the QRS axis shifted to the left. The heart area on x-ray doubled and the muscle glycogen dropped to one third of the initial value. Vacuoles and fatty infiltration occurred in the myocardium. The mortality was 20 per cent. The surviving animals developed rather complete compensation within three to six months; T_1 became positive again but the left axis shift and Q-T prolongation continued; the heart weight doubled but the degenerative myocardial changes disappeared and only diffuse hypertrophy was evident histologically. The glycogen content was restored to normal within 30 to 40 days. In 20 rabbits with removal of a large part of the cerebral cortex 20 days before the production of experimental aortic stenosis, the mortality in the acute decompensation was 50 per cent and the recovery was delayed. The author concludes that the removal of the cerebral cortex interferes with the development of cardiac compensation [Meerson (121)].

A detailed analysis of blood gases in 26 normal subjects and 51 cardiac patients subdivided into three phases of progressive decompensation was made by Georguevskaia (122). Degenerative changes in skeletal muscles were found in 30 patients with chronic cardiac decompensation [Anatenko (123)] as evidence of prolonged peripheral hypoxia. Renal blood flow, studied with the phenol red method, decreased as venous pressure increased [Khodzhamirova (124)]. The mean increase of the venous pressure in the Valsalva maneuver was 125 mm. H_2O in normal subjects and compensated cardiac patients, 70 mm. H_2O in "subcompensated" patients, and 40 mm H_2O in frank decompensation. The venous pressure, instead of increasing with lifting of legs decreased in some decompensated patients [Lotvin (125)].

Ligation of the inferior vena cava unexpectedly decreased leg edema in patients with cardiac decompensation [Rusniak (126)]. Venous occlusion by means of tourniquets around the four limbs in patients decreased the circulating blood volume by about one liter, and decreased also the heart size. In some patients, this procedure stopped paroxysmal tachycardia and atrial fibrillation [Medvedev (127)].

CARDIAC RESUSCITATION

Isolated human heart.—Restoration of activity was successful in 168 out of 240 isolated human hearts [Andreev (128)], removed usually one to one and one-half hrs. after autopsy and kept *in vitro* at 5° to 10°C. for 3 to 131 hrs. In 117 experiments perfusion was started 24 hr. or more after death. The temperature was gradually increased; in most of the revived hearts activity started between 32° and 35°C. The perfusion fluid consisted of Tyrode solution with insulin, thyroxin, ascorbic and nicotinic acids, thiamine, and often human plasma (from 5 to 200 cc. per liter). In some experiments, blood and plasma mixtures were used, but oxygen saturation was sufficient for resuscitation in absence of erythrocytes. The maximum restoration time for activity of the whole heart was 43 hr., of the ventricles alone 50 hr., and of the atria alone 75 hr. after death. Cardiac activity was resumed from 2 min. to 2 hr. after onset of the perfusion, and occasionally even later, with the greatest incidence between 16 and 30 min. Once restored, activity was usually maintained as long as the perfusion was continued, i.e., from one and one half to 13 hrs. The first contractions occurred most frequently in the right atrium spreading to the left atrium, right ventricle and left ventricle, but the sequence was quite variable. The spread of activation from one part to other parts of the heart varied from one min. to two and one half hrs., but in most experiments occurred within 15 min.

Activity of the whole heart was restored in 28 experiments, of three parts in 33, of the ventricles only in 30, and of the atria only in 77. While usually the right atrium was restored earliest, it was refractory after pneumonia, tuberculosis or diphtheria; specific toxic effects of preceding disease were noted also for other parts of the heart; negative results were more frequent in pulmonary and central nervous system diseases. Atropine, epinephrine, and digitalis favored the resuscitation particularly of the left atrium. Small concentrations of alcohol improved the contractions of the whole heart, but had no effect on partial contractions. The percentage of resuscitations declined with the elapse of time after death, and with the age of the patient. The best results were obtained with hearts under five years of age.

During resuscitation, the coronary flow increased from about six to several one hundred cc. per min. The first signs of electrical activity were multifocal slow ventricular complexes of low voltage or ventricular fibrillation; later one ventricular focus became dominant with shortening of the width and increase of voltage of the ventricular complexes. Larger amplitudes occurred first singly or in short faster runs, interrupting the slow oscillations; finally typical QRS and T waves developed. Atrioventricular conduction was only rarely restored. However, there was a large variability in the sequence of patterns during restoration. A relationship between products of the carbohydrate and phosphate metabolism and the electrical activity was demonstrated.

Intra-arterial injection.—Of the various procedures studied for restoration of cardiac activity in advanced shock and cardiac arrest, Negovskii (129)

found intra-arterial centripetal (toward the heart) blood injection to be the most effective. In over 500 animal experiments, death (cardiac and respiratory arrest) was usually produced by hemorrhage and occasionally by asphyxia.

In the agonal phase or after respiratory and cardiac arrest, intra-arterial centripetal injection, together with artificial respiration, and after 10 to 15 sec., injection of 0.2 to 1 cc. epinephrine (1:1000) usually produces a dramatic recovery of cardiac and respiratory activity within 30 to 40 sec.

Blood is superior to blood substitutes, and pulsating injection is more effective than continuous injection. The site of arterial injection is irrelevant, but the brachial (in man reached through a small skin incision) or the femoral artery (in animal experiments) is favored because of easier accessibility.

Local application of novocaine at the site of injection almost abolishes the recovery of cardiac activity which then can be produced by infusion in the same artery closer to the heart. Obviously, the effect is produced through a reflex elicited from the arterial endothelial surface. After section of the vagus or atropine, restoration of cardiovascular function and respiration is very rare [Medvedeva & Pruss (130)]. Dogs with denervated carotid sinus succumb faster to hemorrhage, the period of possible revival is shortened, and restoration of cardiac activity is delayed [Serafimov & Pondimitrov (131)]. Stimulation of the sciatic nerve delayed the time of death in acute hemorrhage from about 8 to about 17 min. in 35 dogs, and accelerated resuscitation in 15 out of 30 animals. [Smirenskaia & Riabov (132)]. Stimulation of the central end of the vagosympathetic nerve accelerated the increase of arterial pressure in the initial phase of resuscitation (129). These data are believed to show involvement of spinal and autonomic pathways in the cardiac reflex elicited from the arterial wall, but the cardiac response to intra-arterial injection could also be obtained after elimination of the central nervous system by ischemia lasting 105 min. [Galperin & Kandel (133)], so that, in addition, an axon reflex seems to be involved. Intra-arterial injection also increases the arterial pressure of normotensive dogs [Kandel (134)]. This reaction depends on the speed, but not volume (5 to 20 cc.) of injection, is abolished by local novocaine, but not by vagotomy and section of the spinal cord at the sixth or seventh cervical segments. One might expect that the reflex could also be elicited with centrifugal arterial injection. This is indeed the case, but it is much less effective (129) and produces often ventricular fibrillation. Centripetal direction is possibly a stronger stimulus, but the greater efficiency of centripetal infusion is probably mainly due to the direct effect on the coronary circulation.

Blood reaches the coronary arteries within 15 sec. after start of the injection of 50 to 80 cc. as shown by means of vital staining in experiments with dogs. With the start of cardiac activity the coronary pressure increases to 25 mm. before a peripheral pulse can be demonstrated, and more rapidly after the appearance of a peripheral pulse, together with the general arterial pressure. In human cadavers, filling of the coronary arteries occurs 3 to 20 sec.

after start of the intra-arterial injection, after 40 to 100 cc. have been given (129).

The effect of intra-arterial and intravenous injection was compared in the agonal phase of prolonged ischemia (2 to 4 hr.) at an arterial pressure of about 30 mm. In contrast to the prompt response to centripetal arterial injection, intravenous injection was not only useless, but produced heart failure, as demonstrated phethysmographically [Zolotokrilina (135)]; larger volumes occasionally produced cardiac arrest. At the same speed and volume of injection in advanced shock (the arterial pressure being 10 mm.), intravenous infusion produced an insignificant increase of the arterial pressure by about 15 mm., together with increased heart volume, while intra-arterial injection increased the arterial pressure to 90 mm. without change of the heart volume [Negovskii (136)].

Resumption of cardiac activity is associated with slow idioventricular rhythm and prolonged Q-T interval, which shortens to a normal duration quite rapidly (129). Hypothermia (at a body temperature of 19° to 29°C.) prolongs the period of "clinical death" from five to six min. up to 60 min. (86, 137, 138). However, resuscitation nearly always produces ventricular fibrillation in hypothermic animals, necessitating use of a defibrillator. In confirmation of Negovskii, Persianinov (139) also found blood superior to blood substitutes given intra-arterially in clinical death or premortal shock (produced by asphyxia), but 40 per cent glucose or 10 per cent calcium chloride were effective at an arterial pressure of 30 mm. or higher. Particularly successful was the combination of calcium chloride and blood. Radionov (140) studied the changes of circulating blood volume after replacement of fatal blood loss with protein-containing blood substitutes; initial decrease was followed seven to eight hr. later by hydremia and increase of the total amount of circulating protein. This reaction was prognostically favorable.

For clinical application in cardiac arrest, intra-arterial centripetal injection of 250 to 500 cc. blood at a pressure of 180 to 200 mm. Hg, is recommended; in advanced shock, less blood and pressure (160 to 180 mm.) is required. Intravenous blood transfusion is added as soon as cardiac activity is resumed, i.e., usually after 30 to 40 sec. Arterial injection is stopped as soon as the arterial pressure in the contralateral arm reaches 90 to 100 mm., usually after injection of 150 to 300 cc. blood. Intra-arterial injection is suggested only in advanced shock. Complications such as air embolus or ventricular fibrillation, are extremely rare.

Clinical experience with combined intra-arterial and intravenous transfusion, as compared with intravenous transfusion alone, is highly encouraging. It decreased the mortality in 1000 cases of severe traumatic shock from 57.6 to 25.5 per cent [Gadjiev (141)]. Of such patients, 68 per cent were fit for surgery after intravenous transfusion compared to 91 per cent after arteriovenous transfusion [Tachmuradov (142)]. In 1253 cases of severe shock who did not respond to other therapeutic procedures, arterial infusion produced complete restoration of arterial pressure in 71.6 per cent, with

ultimate survival of 56.9 per cent, according to the review of Negovskii (129).

The lower ultimate survival rate is attributable to the failure of elimination of the cause of the shock except in hemorrhage, and to secondary myocardial or brain damage. Gurvich & Zolotokrilina (143) found pronounced ECG changes in animals in advanced shock produced by slow hemorrhage. In spite of good response to intra-arterial injection, the animals died in heart failure caused by irreversible changes. Maksimov (144) found the degree and persistence of ECG changes depending on the length of "clinical death."

Detailed attention was given to changes in metabolism and the autonomic and the central nervous system in shock, in cardiac arrest, and in various phases of resuscitation. As an example, Negovskii *et al.* (145) produced cardiac arrest in dogs with established conditioned reflexes. Full recovery of the conditioned reflexes occurred when cardiac arrest lasted not longer than four minutes but the recovery depended not only on the length of anoxia but on individual characteristics of the central nervous system.

CIRCULATION

HEMODYNAMICS

Savitskii's monograph (146) is a critical comprehensive review of the most commonly used functional hemodynamic methods, with a thorough analysis of the mathematical and physical basis. Of particular interest is his new method of "tacho-oscillography," based on measurement of the speed of volume changes of the brachial artery in the pressure cuff recorded by means of a differential manometer simultaneously with the radial artery pulse. In addition to diastolic, mean, and systolic pressure, this method permits measurement of the kinetic pressure component, which was verified in model and animal experiments. Measurement of the kinetic pressure component was of prognostic significance for patients with arterial hypertension.

Nikitin (147) used Savitskii's tacho-oscillography together with measurement of cardiac stroke volume and arterial pulse wave velocity by means of Broemser and Ranke's method. The normal limits of the ratio of pulse wave velocity in muscular arteries "Cm" (measured between the subclavian and radial arteries) to that in elastic arteries "Ce" (measured between the carotid and femoral arteries) ranged from 1.11 to 1.30 in 137 normal subjects. This ratio has the advantage of eliminating the arterial pressure as reference for the pulse wave velocity. It was substantially decreased in patients with arterial hypertension (0.5 to 1.1), except in the earliest phase, mainly because of a relative decrease of the tone in muscular arteries, which is probably a functional compensation phenomenon. Nitroglycerine produced a further decrease of the ratio Cm:Ce in hypertensive patients. A simple method for measuring the pulse wave velocity was suggested by Busygin & Hefedov (148).

Atherosclerosis of the aorta was found to be more important for the in-

crease of pulse wave velocity than hypertension, according to Miasnikov (149) who compared patients with atherosclerosis of the aorta demonstrated by x-ray, with arterial hypertension, and with combinations of these conditions. In 36 patients with arterial hypotension, the minute volume, determined with Broemser and Ranke's method, was increased but not sufficiently to compensate for the decreased peripheral resistance [Silvestrov (150)].

The greater arm-to-arm circulation time, measured by means of radioactive sodium, in cardiac decompensation confirms older findings with more subjective methods. It was found to be increased also in clinically compensated patients with valvular heart disease [Fateeva & Maslova (151), Bobkova *et al.* (152)]. In arterial hypertension it increases progressively with the duration of disease and the arterial pressure level [Fateeva *et al.* (153, 154)]. The pulmonary circulation time, determined as difference between two radiation peaks, interpreted as arrival in the right and left ventricle (normal mean, 6.2 sec.), also increased with progressive arterial hypertension up to a mean of 9.2 sec. for the advanced phase (155).

CEREBRAL CIRCULATION

Cerebral ischemia.—One or two minutes after ligation of the anterior and middle cerebral artery near the circle of Willis in dogs and cats, the ischemic region became pale, the lumina of all arteries decreased together with a drop of arterial pressure, and the venous flow occasionally reversed its direction [Kosmarskaia (156)]. Several minutes later, the arteries started to enlarge again dependent on the intraluminal pressure; collaterals did not open below a critical pressure. Even under the best circumstances the collateral circulation was inadequate to prevent edema and cerebral degeneration in the ischemic region.

After successive ligation of the carotid and vertebral arteries at different time intervals, dogs developed the best collateral circulation followed by cats, rabbits, white rats, and white mice in that order. The general behavior of the animals, pain sensitivity, pupillary reflex, body temperature, respiration, and mortality served as criteria of brain functional capacity. In dogs, simultaneous ligation of both common carotid arteries and both vertebral arteries did not produce symptoms of acute cerebral ischemia, while in mice, ligation of the second carotid artery, 13 to 42 days after ligation of the first one, produced nearly 100 per cent mortality. It was concluded that the potential development of collateral circulation improves with the development of the central nervous system [Vasilev (157)]. Lethal asphyxia increased the intracranial blood content by about 100 per cent of 132 cats, except in the age group from three to ten days [Zhukova (158)].

The effect of acute cerebral ischemia on reflex activity was studied by Kudrin (159) in frogs. The more complex reflexes disappeared earliest and recovered latest after restoration of circulation. Urethane, chloral hydrate, and barbital sodium accelerated the abolishment of reflexes in ischemia and delayed their recovery.

Subcutaneous injection of a "therapeutic complex" consisting of urethane, barbital, glucose, ascorbic acid, vitamins A and B, combined with breathing of a 50 per cent oxygen mixture, decreased the mortality and the degenerative changes in the brain in cerebral ischemia, produced in rats by carotid ligation [Petrov (160)]. Since the "therapeutic complex" contains urethane and barbital, the results of the two series (159, 160) seem to be somewhat at variance, but this may be attributable to the different animals used.

In lethal acute myocardial infarct in man, vascular changes in the brain, with resulting damage to brain tissue, are always present, probably because of secondary cerebral ischemia [Melikova (161)]. Degenerative changes in the cerebral cortex and medulla oblongata were also frequent in patients with cor pulmonale [Zlatoverov (162)] and believed to be due to the increase of venous pressure. Compression of the jugular vein increased the threshold of the conjunctival reflex, more so in patients with disturbance of cerebral circulation or in right heart failure than in normal subjects (162).

Mechanical recording.—Patients with traumatic bone defects in the skull offered an unusual opportunity for comparison of intracranial and forearm circulation recorded pneumographically [Bentelev (163, 164)]. Breathholding in the expiratory and inspiratory positions with a drop of arterial oxygen saturation from 19.5 to 15 vol. per cent increased the volume and pulsations of the intracranial circulation, while the forearm circulation decreased in expiratory position and increased slightly in inspiratory position. After resumption of breathing, a decrease of forearm volume and pulsations contrasted with the continued elevation of intracranial circulation. Breathing of a 7 per cent CO_2 -93 per cent O_2 mixture increased and 10 per cent O_2 -90 per cent N_2 decreased intracranial pulsations and volume. During the Mueller test and during the Valsalva procedure, the decrease and the increase in volume respectively were more pronounced in the forearm than in the intracranial circulation. The greater stability of the intracranial circulation is believed to be due to the rigidity of the skull. Discrepancies between intracranial and forearm circulation were also noted during and after hyperventilation, and in sleep in which there appears an increase of intracranial and a decrease of forearm circulation. Diuretin, a theobromine-salicylate mixture, in dosage of (0.5 gm) produced a four fold increase of the amplitude of intracranial pulsations without essential change in arterial pressure [Bentelev (164)].

Impedance plethysmography.—The extensive work of Naumenko, Kedrov and associates with impedance plethysmography is an outstanding contribution to the dynamics of cerebral circulation (165). This method, used with electrodes sealed into the cranium, is ideally suited for the study of instantaneous changes of intracranial circulation in animals although the total cerebral blood flow is not obtained. The electrodes were usually placed between bone and dura mater, but occasionally inserted into various parts of the brain for measurement of regional cerebral circulation. The greatest density of the electrical field inside the skull was found in the spaces filled

with cerebrospinal fluid; the difference of the electrical conductivity between fluid and brain tissue was greater in living animals than in cadavers. The effect of the frequency of the alternating current was studied up to 100,000 c.p.s. (166). With an external resistance of 300 ohm, a change of 1/10 ohm deflected the galvanometer deflection 60 mm. The variations with pulsations varied between 1/10 and 1/100 ohm. Scalp electrodes do not record intracranial circulation because of the low bone conductivity. Since cerebrospinal fluid has an electrical conductivity approximately twice that of blood, and blood a higher conductivity than tissues, the directional changes attributable to blood inflow in other regions and to fluid displacement by blood are opposite. Fluid displacement is important for slow base line changes, but cannot account for the rapid beat to beat pulsations, which, in the absence of volume changes in the rigid enclosure of the bony skull, result from resistance variations with blood flow velocity. This was directly shown with recording from rigid tubes inserted in veins leaving the cranial cavity. The contour of the cerebral venous pulse resembled that of an arterial pulse with a dicrotic notch.

Asphyxia produced an increase in the amplitudes of intracranial and cerebral vein pulsations of 10 to 15 times the resting value, elsewhere in the body the increase was much smaller or absent. This was confirmed with direct measurement of the cerebral venous outflow by Kedrov *et al.* (167). The authors conclude that the pulsations of intracranial arteries are directly transmitted to the veins, bypassing the capillaries, which is a general pressure effect and not a proximity effect of the artery to the closest veins. Therefore, pulse recording in veins leaving the cranial cavity is a fairly reliable indirect method for study of the intracranial circulation. The amplitude of the venous pulses is smaller than that of the intracranial pulsations because of the greater volume capacity of the venous bed. The closed skull is a characteristic determinant of the cerebral circulation; even a small aperture doubled the amplitude of intracranial and cerebral venous pulsations. Because of the direct arterial to venous pressure transmission, the pulse fluctuations of the intracranial pressure are very small (1 to 2 mm. H₂O), as shown with three different methods (changes of condenser capacity, electromagnetic induction, piezocrystal resistance) [Naumenko (168)]. The effects of sympathetic or vagal stimulation (vasoconstriction or dilatation, respectively), and of epinephrine on the intracranial circulation are quite small (approximately 50 per cent of the original amplitude) as compared with the change in extracranial or limb circulation (165, 169). The specific effect of carbon dioxide on cerebral circulation occurs also with clamped carotid arteries, excluding the carotid sinus as mediator. Increase of the arterial pressure plays only a minor part in the effect of carbon dioxide on intracranial circulation, as shown by comparison with the elevation of arterial pressure produced by clamping of the abdominal aorta. In contrast, the increase of amplitudes after epinephrine injection parallels the increase of arterial pressure; after clamping the carotid arteries, epinephrine decreases the amplitude; it also diminishes the effect of asphyxia on the cerebral circulation. The increase of amplitude after

acetylcholine shows a regulatory mechanism for the maintenance of cerebral circulation in spite of the drop of arterial pressure. Under the influence of carbon dioxide cerebrospinal fluid is replaced by blood, as interpreted from changes of the base line, which is in good agreement with Zhukova's findings (158). There was no correlation between the changes of the baseline and amplitudes.

The effects of caffeine, histamine, nitroglycerin, alcohol, nicotinic acid, reactive hyperemia, and stimulation of the sciatic nerve were used in interpretation of the mechanism of headaches in arterial hypertension by Kedrov & Naumenko (165, 170, 171).

Thermoelectric recording.—Blood flow in various parts of the brain was estimated from temperature variations recorded from inserted thermoelectrodes. Mechanical stimulation of the intestinal wall by inflation of a rubber balloon increased the blood flow in the hypothalamus, the dura and pia mater parallel with the increase of arterial pressure in 22 out of 34 dogs but in some animals cerebral vasoconstriction occurred at the same increase of arterial pressure [Ryzhova (172)].

CORONARY CIRCULATION

Coronary blood flow was measured in dogs with implanted thermoelectrodes in chronic experiments by Marshak & Aronova (173). The flow was increased by meals, breathing of 2 to 3 per cent carbon dioxide, mechanical stimulation of the rectum by means of an inflated rubber balloon, and by static work (load of 5 to 8 kg. on the back). Pain, produced by electrical skin stimulation, first decreased and later increased the coronary flow. Emotional disturbance (fear) decreased the coronary blood flow, which is an important experimental documentation of the potential role of emotional upsets in the production of angina pectoris.

Of great interest is the direct visual observation of coronary vessels through a healed-in window in the chest wall in chronic experiments in dogs [Sinitsyn (174)]. Using a strong light source, changes of the diameter of coronary vessels were recorded cinematographically at the speed of 24 frames per sec. and measured on a screen. The diameter of the large veins increased 40 per cent and that of the arteries 20 per cent in diastole, when small venous loops and anastomoses appeared [Andreev *et al.* (175)].

Epinephrine, ephedrine, caffeine, and atropine, usually dilated the coronary arteries, but occasionally constricted. The epinephrine dilatation was most consistent, but fluctuations of the diameter were common. The largest dilatation varied from 0.9 to 1.8 mm. By comparison, injection of 0.9 per cent sodium chloride produced fluctuations of only 0.05 mm. The response to epinephrine, ephedrine, and nitroglycerine was rapid, and slower (maximum dilatation appearing between 6 and 30 min.), but more prolonged (up to 80 min.) after caffeine. During dilatation the P wave usually increased and the T wave decreased or became occasionally inverted, with reverse changes during constriction [Trofimova (176, 177)].

Detailed histological studies of coronary vessels in man and animals were

summarized in a monograph by Ognev *et al.* (178) which is, with its many excellent illustrations, one of the best sources available. In contrast to other authors, spasm of coronary arteries is held to be a distinct possibility. After coronary occlusion, collaterals develop, not only from the coronary arteries, but also from the net of arteries in the diaphragm and pericardium, supplying the region around the heart. On the basis of their studies, the authors suggest a new type of operation for collateral vascularization in patients with coronary disease. The large normal variability in diameter, the ramification and anastomoses of coronary vessels explains, perhaps, the large individual difference in developing clinical coronary disease in advanced atherosclerosis.

PULMONARY CIRCULATION

Direct visual observation through a window in the lateral chest wall was used also for the study of pulmonary circulation in cats and rabbits. Capillaries and arterioles were easily visible. The blood flow was quite variable; the arterial pulsations were more pronounced with slower flow. The blood flow in veins is usually uniform, but occasionally pulsating, though less than in arteries. In developing heart failure, the flow stops first in the veins, and in more advanced decompensation, all visible capillaries disappear. In short cardiac arrest, the direction of blood flow may be reversed [Bekauri *et al.* (179)].

UNCONDITIONED AND CONDITIONED VASCULAR REFLEXES IN MAN

The development of a fairly well standardized method for the study of conditioned and unconditioned vascular reflexes in man is one of the major achievements in cardiovascular physiology in the U.S.S.R. As compared with conditioned motor responses, conditioned vascular reflexes have the great advantage of eliminating the voluntary component. Thus, functional characteristics of the central nervous system and their involvement in cardiovascular regulation have become accessible to quantitative measurement. Since fundamental environmental variables are used as conditioned and unconditioned stimuli, the results have a bearing on the important question of environmental adjustment in normal and pathologic conditions.

The method is used by most authors in the following way: a volume plethysmogram is recorded from one forearm or hand before and during application of cold or heat to the contralateral arm (immersion into water from 0° to 5°C. or about 45°C., respectively). In normal subjects, a prompt reflex vasoconstriction to cold and dilatation to heat occurs. The cold or heat application (unconditioned stimulus) is preceded by an acoustic or optic (conditioned) signal, and conditioned reflexes are obtained after a certain number of repetitions. Usually the conditioned reflex is identical with or similar to the unconditioned reflex. On this basis differential inhibition is developed between a positive and a negative signal (for instance between green and red light). Furthermore, these stimuli are substituted by verbal signals. Finally, the speed of extinction is measured (number of trials without reinforcement until

disappearance of the conditioned response). With this procedure the following criteria are obtained: resting fluctuation of the plethysmogram, latent period for conditioned and unconditioned response, amplitude, and duration of the response, number of repetitions (a) for development of the conditioned response, (b) for conditioned differential inhibition, and (c) for extinction, the latter being an index of the stability of the reflex. Further criteria are obtained from the relationship of the responses to the strength and type of the various conditioned and unconditioned stimuli. The variety of changes in the different criteria is too large to be reviewed in detail. For condensation of presentation the following fundamental types of an abnormal reaction are differentiated: (a) absent or hyporeactive (slow in development, long latent period, small amplitude); (b) hyperreactive (opposite in direction to the first); (c) paradoxical (constriction to heat application or dilatation to cold application); (d) stable-unstable size of fluctuations of baseline, extinction of conditioned responses.

Most of the authors used volume-pneumographic recording, but several modifications were suggested such as pneumoelectric recording [Babskii (180)], pneumooptical recording [Ilinskii (181)], and photoelectric recording [Verner (182)]. Important suggestions for standardization of the procedure were made by Okhnianskaia (183), Kondrat'ev (184), and Iakovlev (185).

Normal subjects, and physiological stresses.—The effect of stimuli other than thermal was also explored. Conditioned vasoconstriction to breathing of 10 to 12 per cent CO₂ and to pain developed faster (two to six repetitions) and was more stable than that to cold application [Lamberg (186)]. Mental work increased these conditioned reflexes; physical work decreased the conditioned and increased the unconditioned reflexes in normal subjects (186). Mental work (calculations) alone produced vasoconstriction [Valdman (187)], and a stable conditioned reflex could be developed in 15 young subjects on this basis (association of mental work with various sensory stimuli). A differential inhibition to different acoustic stimuli was obtained after 8 to 14 repetitions [Petelina (188)]. Conditioned vascular reflexes to cold were associated with a conditioned increase of oxygen consumption [Isaakian (189)]. Conditioned vascular reflexes were obtained to maximum respiration, associated with optic, acoustic, and verbal stimuli [Okhnianskaia (190)]. Vascular reflexes were produced by mechanical and thermal stimulation of the vagina; the effect decreased after the menopause [Akhmedova (191)].

Auditory, visual, and thermal stimuli produced initially opposite reactions by the vessels of the head (dilatation) and hand (constriction), but thermoregulatory reactions (vasoconstriction on cold and dilatation on heat application) became uniform in hand and head after repetition. Pain stimuli produced uniform vasoconstriction [Vinogradova & Sokolov (192)].

In a sleepy state produced by monotonous sound a paradoxical reaction to the conditioned as well as unconditioned stimulus developed: peripheral vasoconstriction to the heat stimulus and peripheral vasodilatation to the

cold stimulus. When the subject was wide awake, the amplitude of the respiratory movements and the peripheral pulse volume were fairly stable, but when he was dreaming, large slow phasic synchronous oscillations with a cycle length of about one minute appeared in the plethysmogram, along with an increase of respiratory movements and a decrease of the forearm volume [Eremin & Chernikov (193)].

In most children from two to five years, fairly stable conditioned vascular reflexes were readily obtained, but differential inhibition was poor; in a minority the conditioned reflexes were inert and unstable, according to Tonkova-Iampolskaia (194).

Acceleration in a centrifuge decreased the unconditioned and conditioned vascular reflexes to cold application and interfered with the differential inhibition [Usachev (195)]. In some of the 35 subjects the response was abolished or become paradoxical in "extreme" acceleration (actual g values were not given).

Patients with cardiovascular disease.—The majority of 56 patients with coronary insufficiency showed hyporeactive or abolished unconditioned and conditioned vascular reflexes, particularly in periods of frequent angina attacks. The minority was hyperreactive, with delayed extinction and large spontaneous fluctuations [Zoslavaskaia (196)]. Exaggerated fluctuations, either spontaneous or as after-effects of conditioned reflexes, were frequently observed also in another series of 46 patients [Iakushkevichus (197)]. In 23 patients of this group, no differential inhibition could be obtained. Frequently, heat as well as cold produce unconditioned and conditioned vasoconstriction. In the first days of acute myocardial infarction, the unconditioned as well as the conditioned response to cold were abolished, but dilatation to heat was maintained [Gleser (198)]. The author interpreted this phenomenon as a defense mechanism and concluded that coronary insufficiency is not a local myocardial process, but involves a disturbance of general circulatory regulation.

In order to study possible right-left asymmetry in patients with arterial hypertension the thermal stimuli were applied to the spine, and drinking of hot and cold water was added to the series of unconditioned stimuli. In the initial phase of arterial hypertension, the reactions of the right and left arm were usually identical. Conditioned reflexes to acoustic, optic and verbal stimuli developed rapidly (four to six repetitions) and there was a tendency to more pronounced reactions to cold than to heat, but, in general, the disturbance was slight. With progressive disease, conditioned and unconditioned reflexes became hyporeactive [Rybkin & Segal (199), Kostiukhina (200)]. Occasionally paradoxical reactions were obtained. With clinical improvement produced in one series by diathermy treatment of the carotid sinus (200), the vascular reactions approached normality (199, 200). However, the vasoconstriction during mental calculation was usually not affected by treatment.

In acute rheumatic heart disease (RHD) unconditioned and conditioned vascular reflexes became hyporeactive in proportion to the clinical severity,

according to Danenkov (201). In the chronic phase of this disease, the response to cold was maintained in 32 out of 35 patients, while the response to heat was abnormal in all patients (paradoxical in 12, diphasic in 8, absent in 15). It may be suggested that patients with RHD might be handicapped in adjustment to hot environment.

Abnormal vascular reflexes (diphasic, paradoxical or absent) were found in the majority of patients with arterial hypotension by Sosiura & Aksent'ev (202) and Silvestrov (203), who found the same to be true for 31 patients with neurocirculatory asthenia (204). The suggestion of exaggerated inhibition of vascular reactions was supported by EEG records (203).

Diseases of the central nervous system.—Unconditioned and conditioned vascular reflexes were disturbed in the paranoid form of schizophrenia [Segal (205)]. In another group of schizophrenic patients, the response to nitroglycerin (an initial constriction followed by a prolonged dilatation and the appearance of respiratory variations in the forearm plethysmogram) was used as basis for conditioned reflexes. Their formation was slow, and the fast extinction inhibition also suppressed the unconditioned response [Morosov (206)]. In patients with neurasthenia, the response to cold was diminished, and in 6 out of 22 patients heat dilatation was preceded by initial constriction [Blagosklonnaia *et al.* (207)]. Profound disturbances of vascular responses were observed in hypochondriac psychoses; conditioned as well unconditioned responses were easily fatigued on repetition. The conditioned vascular reactions were hyporeactive, unstable and occasionally unobtainable. Paradoxical reactions occurred in several patients [Kogom (208)]. Instead of thermal stimuli, the vasoconstriction produced by salt, acid, and bitter tastes was used as unconditioned response in 49 patients with hemiplegia. Unconditioned and conditioned responses were more pronounced on the normal side [Rolle (209)]. Large spontaneous fluctuations of the hand plethysmogram in 36 patients with neurosis disappeared in sleep induced by drugs or hypnosis. Absence of pain or noise sensation suggested in hypnosis abolished the vasoconstriction to electrical skin stimulation and noise of 80 to 100 decibels [Povorinskii (210)].

In the majority of 43 patients with various central nervous system disorders, particularly in patients with encephalitis, mental calculations produced vasodilatation instead of the normal vasoconstriction [Ogienko (211)].

Miscellaneous diseases.—Normal conditioned vascular reflexes were only rarely obtained in 30 patients with thyrotoxicosis, although there was a large variety of abnormal deviations. Paradoxical reactions were frequent. Surgical treatment caused first the unconditioned and later the conditioned reflexes to become normal [Amiragova (212)]. Inert vascular responses, and right-left asymmetry were frequent in patients with liver disease [Kononov (213)]. In the acute type of gastric ulcer, characterized by severe episodes with fast remissions, the vascular reactions were hyperreactive; conditioned reflexes were obtained after as few as two to four repetitions, and the extinction was slow. In patients with the chronic type of gastric ulcer (with less violent, but

more prolonged episodes), the conditioned reflexes were unstable and often paradoxical [Il'inskii (214)]. In 11 patients with skin diseases, the conditioned vascular reflexes were normal, but often increased in hypnotically induced sleep [Dumova (215)]. Abnormal vascular reactions were common in 65 patients with acute dysentery, the inert and paradoxical type of response prevailing in the more toxic patients [Bunin (216)], or in patients with chronic dysentery developing secondary malnutrition [Kortev (217)]. Hyporeactive responses were typical in the common cold ("grippe") and in 60 patients with typhus abdominalis, but the results in another series of 60 patients with dysentery, typhus exanthematosus, and typhus abdominalis are somewhat at variance [Bunin (218)]. The conditioned reflex was weak in 12 anemic patients after hemorrhage, and prolonged in 19 patients with endarteritis obliterans; but conditioned reflexes were obtained in all 31 patients [Tilis & Ishanova (219)]. Finger volume plethysmography was used as an index of peripheral circulation in spinal anesthesia, during surgery, and in shock [Karpilovski (220)].

In summary, the work demonstrates disturbance of unconditioned and conditioned vascular responses in man in various physiological stresses and diseases. This principle deserves consideration in the total clinical picture, and has been featured here because of its novelty. Of course, other methods were also used for the study of peripheral circulation; for instance, skin temperature measurement in endarteritis obliterans [Petkevich (221)], in arterial hypertension [Gelfer (222)] and after sympathectomy [Ass (223)], or reactive hyperemia in endarteritis obliterans [Shamova (224)].

Mental work increased the pulse amplitude, piezoelectrically recorded, in the temporal and decreased it in the radial artery of 25 normal subjects. These changes were exaggerated in patients with early arterial hypertension. In patients with cerebral concussion, mental work decreased the temporal and increased the radial pulse amplitude (paradoxical reaction) [Greenshtein & Bragina (225)]. (The extensive studies on cardiovascular reflexes in animals could not be included because of space limitation.)

PHYSICAL WORK

Training.—The minute and stroke volumes of ten well trained athletes at rest and during maximum bicycle ergometer work were higher than those of nine untrained subjects, this finding confirming accepted concepts [Markovskaia (226)]. The maximum attainable heart rate of athletes ranged from 200 to 228, that of the untrained subjects from 180 to 190 beats per min. In 52 men trained in various types of athletics, the systolic pressure in the work arm (usually the right in tennis players, etc.) was higher at rest, and the drop after exercise greater than in the other arm [Makarov (227)]. In trained athletes, the heart rate increased in anticipation of exercise and was associated with increased amplitude of oscillations of the transverse heart diameter in x-ray kymograms [Salina (228)]. Only 9 of 28 athletes showed a resting bradycardia, which is generally considered to be typical for

the state of training [Salina (229)]. Prolonged training of one to three years duration in 126 male adolescents of 14 to 18 years of age had no effect on arterial pressure [Buchenko (230)]. Training in standardized exercise decreased blood lactate, pulmonary ventilation, and oxygen consumption in only one half of the subjects [Nesbetaeva (231)]. It appears that effects of physical training are found to be less consistent in the U.S.S.R. than elsewhere.

Exercise tolerance tests.—As in other countries, the ECG response to exercise (discussed above) seems to have replaced other functional exercise tests in the examination of cardiac patients. In patients with circulatory insufficiency the increase of the venous pressure was greater and the recovery slower than in normal subjects, but rhythmic deep pulmonary ventilation accelerated the recovery [Poroikova (232)]. The effect of mild gymnastic exercise on the mechanics of heart movement was studied with Babskii's method (109) in 28 patients with subacute myocardial infarction, but the results were not consistent [Karpman & Voskanov (233)].

Therapeutic use of exercise.—A combination of various types of mild to moderate exercise was used in the treatment of 52 patients with heart disease, mostly valvular lesions and cor pulmonale, in general with favorable results [Poroikova (234)]. Very mild exercise, started about three weeks after myocardial infarction, with gradually increasing load improved the clinical condition and the response to an exercise tolerance test in most patients [Kareva & Markov (235)]. In 130 cases of myocardial infarct Nechaev & Rozanov (236) also reported favorable results of exercise therapy.

Work capacity of cardiovascular patients.—Work experience, extending over six years of observation, in 200 rehabilitated patients with myocardial infarction was surprisingly good; 76 per cent resumed their previous job, 14 per cent changed the occupation because of limited capacity, and only 10 per cent were invalidated [Karamyshev (237)]. In 57 per cent of the working patients no significant ECG changes occurred from morning to evening; in 35 per cent negative T waves in lead I, II or III became isoelectric, which was, possibly erroneously, interpreted as improvement during work; and in 8 per cent the T waves became more inverted. The favorable results were confirmed in another series of 100 patients with myocardial infarction [Karamyshev (238)]. In the majority of 848 patients with arterial hypertension, physically moderate occupational work decreased the arterial pressure by 10 to 15 mm. in the day shift, but increased it slightly in the evening or night shift [Fogelson (239), Farber (240)]. Occupational work involving nervous tension in 213 telephone switchboard operators tended to increase the arterial pressure (239), and the incidence of arterial hypertension in occupations involving nervous tension was 6.7 per cent of 5200 workers compared to 3.7 per cent in workers engaged in physical work (240). Increase at the arterial pressure of hypertensive patients in work involving nervous tension was confirmed by Il'ina (241). It dropped significantly during light to moderate physical work at high environmental temperatures (28 to 35°C.), but the changes in heavy indus-

trial work at high environmental temperatures were not uniform. These field observations were supplemented by experimental exposure of hypertensive patients to 35°C. in a climatic chamber, in resting condition and in standardized exercise. The results were, in general, concordant (241).

Work experience with patients with valvular heart disease in the textile industry was in general favorable [Aleutskaia (242)]. In 47 patients with heart trauma (embedded bullets) prolonged observations revealed no handicap in sedentary or light to moderate physical work [Sosenkii (243)]. Simultaneous electromyograms from the triceps and electrocardiograms (bipolar chest-back lead) recorded with a two channel instrument during heavy industrial work were remarkably free of interference [Vobolazskii (244)].

In animal experiments involving exercise, Miasnikov's (149) results are of particular interest. Running for five minutes on a treadmill produced no fatigue and no myocardial necrosis in normal rabbits, while in rabbits with experimental hypercholesteremia and some lipide deposits in aorta and in coronary arteries, fatigue, and fairly extensive myocardial necroses occurred after a run of three minutes, and repeated work produced changes resembling myocardial infarction.

LYMPH FLOW

Thoracic duct.—In careful experiments, the lymph flow collected from the thoracic duct (T.D.) was much less than the sum of flow from several organs [Rusniak (245)]. In a typical experiment in one dog the combined lymph flow for liver, heart, hind legs, and kidneys was 0.9 cc per min., that of the T.D. was 0.4 cc. per min. After slow injection of 0.5 per cent congo red and 0.5 per cent paraaminohippuric acid into a peripheral lymph vessel the color concentration in the T.D. was low; 12 per cent of the hippuric acid are recovered in urine, 7 per cent in the T.D., and consequently 81 per cent accumulated in lymph nodes and vessels. The pressure in the T.D. increases parallel with that in the superior but not the inferior vena cava (245). However, an increase of the thoracic lymph flow with increase of the pressure in the pulmonary artery, in the portal vein and inferior cava was noted by Petrovskii (246) and by Smirnov (247). An augmentation of the thoracic lymph flow occurred also with increased pressure in the intestinal vessels (produced by venous occlusion or high perfusion pressure up to 240 mm. Hg), in the urinary bladder and kidney (injection of 20 to 100 cc. of 0.9 per cent sodium chloride into both ureters). This response was maintained after section of the vagus nerve and the sympathetic trunks near the diaphragm, but abolished by additional removal of the stellar ganglion [Kokhannia (248)]. Elevation of the intra-arterial pressure up to 100 mm. Hg in [the left lung of dogs isolated *in situ* produced a drop in the arterial pressure and increase in the perfusion flow (Locke's solution) through the T.D. Decrease of the pulmonary arterial pressure produced the reverse changes. Experimental pulmonary embolus decreased the arterial blood pressure and increased the lymph flow [Smirnov (249)]. The changes of lymph flow with the pressure in

the pulmonary arteries were not affected by unilateral vagotomy, but were diminished after bilateral vagotomy. The increased lymph flow with increased pulmonary arterial pressure is believed to facilitate the shift of blood from the pulmonary to the systemic circuit. The view that lymph vessels participate in general circulatory regulations is shared by Kovanov (250). Unilateral compression of one common carotid artery increased the arterial pressure and decreased the lymph flow by 20 to 50 per cent preventing a shift of fluid from blood to lymph vessels. Involvement of the carotid sinus is shown by reproduction of the same result with electrical stimulation of this region, a finding which was confirmed by Vasil'chenko (251). Sympathectomy increased the lymph flow (245).

Peripheral lymph vessels.—Three to eight minutes after subcutaneous injection of 10 cc. contrast solution in the hind legs, filling of lymph vessels was demonstrated with x-ray, which could be prevented by stimulation of the sympathetic trunk (245). The effect of sympathetic stimulation was abolished by denervation of the femoral artery, but not by section of the sciatic or femoral nerve. Injection of epinephrine or ephedrine, and arterial, or venous ligation did not interfere with the filling of lymph vessels.

Ligation of the efferent lymph vessels of the heart produced in dogs myocardial degeneration similar to that of acute myocarditis, together with abnormal changes in the ST segment and the T wave. Ligation of efferent lymph vessels of the kidney together with the ureter accelerated the development of hydronephrosis, and ligation of hepatic lymph vessels together with ligation of the common bile duct produced much greater degenerative changes than ligation of the duct alone (245). Ligation of all visible veins in the legs of dogs, cats, and rats did not produce edema, but after intravenous injection of irritating substances thrombophlebitis with edema (162) developed, a fact suggesting that the peripheral edema is not due to mechanical obstruction alone.

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RESPIRATION¹

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Respiratory physiologists are proud that two of their colleagues, Cour-nand and Richards, were awarded with Forssman a share of the Nobel Prize in Medicine and Physiology for 1956. Their *Nobel Lectures* (60, 160) give an excellent review of the physiological background of their research, and of some special applications to the pulmonary circulation.

MECHANICS OF BREATHING

An excellent review by Radford (156) discusses problems related to our understanding of the mechanical properties of the lungs. The intricate anatomical arrangement of the elastic elements of the lungs and the complex interaction of true elastic forces and surface forces are emphasized.

Inertance.—The conclusion reached by Rohrer in 1915, on the basis of calculations from anatomical data, that inertial forces are relatively unimportant in breathing has been experimentally tested in two laboratories by two different methods. Both Mead (130) and Dubois, *et al.* (75, 40) find that inertance is indeed negligible in comparison with resistance and elastance in any physiological situation. The latter investigators, using forced oscillations, found the chest-lung system of both men and cats to have a natural frequency in the range 7–15 c.p.s. but to be so highly damped that uncontrolled vibrations are prevented. Mead's measurements, made during natural rapid shallow breathing, gave an average value in man for inertance of the lungs and gas stream of 0.01 cm. H₂O/l./sec.² at ordinary barometric pressures. Mead also made measurements at increased ambient pressures up to four atmospheres, and since he found inertance to increase with pressure, he concluded that most of the inertance was in the air stream.

Resistance.—Total flow resistance has been separated into its airway and tissue components from measurements made by Marshall & DuBois (127, 128) on men and by Brody & DuBois (39) on cats by a method involving body plethysmography, and which does not require the use of foreign gases. Tissue resistance comprises about one-sixth the total resistance in the lungs of normal men (127). In some pulmonary diseases a moderate increase in tissue resistance was found (128). Resistance is higher in the supine than in the sitting position [Attinger, Monroe & Segal (10)]. Patients with emphysema and cardiopulmonary disease have an elevated resistance, especially during the expiratory phase [Attinger, Herschfus & Segal (9)]. The average resistance of a newborn 3 kg. infant breathing quietly is about 30 cm.H₂O/l./sec. [Cook *et al.* (58)].

¹ The survey of literature pertaining to this review was completed in June, 1957.

At a simulated depth of 99 ft. resistance is approximately doubled [Marshall, Lanphier & DuBois (129)]. The " K "'s of the simple equation $P = K'\dot{V} + K''\dot{V}^2$ are not constant from depth to depth. This means that the relative amounts of streamline and turbulent flow change with gas density.

Compliance.—A number of studies have been devoted to the measurement of the compliance of the lungs and chest in human subjects. The average compliance of the lungs in 70 young adults as measured by a volume-step technique was found by Frank *et al.* (84) to be 0.165 l./cm. H_2O ; these results compared favorably with those obtained during continuous breathing. Compliance was found to correlate with height, body surface area, and vital capacity but not with sex or age. Heaf & Prime (104) describe a method employing pressure breathing. By use of an esophageal balloon compliance was separated into chest wall and lung components, with the following average results in a group of normal subjects: 0.191, 0.189, and 0.093 l./cm. H_2O for lungs, chest wall, and lungs-plus-chest, respectively. In 44 completely relaxed anesthetized patients Butler & Smith (45) found average compliance for lungs, chest, and lungs-plus-chest to be 0.153, 0.268 and 0.098 l./cm. H_2O . Compliance in anesthetized paralyzed subjects was also studied by Howell & Peckett (109). With static positive pressure inflation, compliance of lungs-plus-chest was 0.078 l./cm/ H_2O , but with cyclic positive pressure inflation it dropped to 0.057 l./cm. H_2O . By the latter method compliance of lungs and thorax was 0.084 and 0.174 l./cm. H_2O respectively. The low compliance obtained by cyclic inflation may arise from unevenness in distribution of ventilation. The observations of Massion (157) of the effects of curare on pulmonary compliance in anesthetized dogs may be pertinent to the findings just summarized.

Pulmonary compliance of a newborn infant is about five ml./cm. H_2O [Cook *et al.* (58)]. It is lower in the supine than in the sitting position [Attinger, Monroe & Segal (10)]. In patients with emphysema and cardiopulmonary disease pulmonary compliance is lower than normal and decreases with increasing frequency of breathing [Attinger, Herschfus & Segal (9); Saxton, and his co-workers (167)]. Brief application of pressure around the body produced by submersion in water or inflation of a G suit causes pulmonary compliance to decrease markedly; this is apparently due to pulmonary vascular engorgement [Bondurant, Hickam & Isley (29)]. In unilateral bronchial occlusion the chest wall compliance on the open side is effectively increased because the mediastinum can invade the closed side [Dale & Rahn (67)].

Attinger *et al.* (10) have observed that the pressure differences recorded from an esophageal balloon are usually less than the simultaneously recorded intrapleural pressure fluctuations and that end expiratory esophageal pressure is generally more positive than the corresponding intrapleural pressure. Farhi, Otis & Proctor (78) found that intrapleural pressure measured simultaneously at various points over the surface of lungs in dogs was essentially identical except at the apices and in the diaphragmatic sinus.

Fry *et al.* (86) have done an excellent and valuable piece of work in evaluating the characteristics of three types of respiratory flow meters (pneumotachographs). All types studied have a good dynamic response up to 34 c.p.s. The harmonic content of flow curves for normal breathing, vital capacity and maximal breathing was also determined in the same laboratory (135). All these breathing maneuvers will be faithfully recorded by a flow meter which responds to 26 c.p.s. Fleisch, the father of pneumotachography, describes a new pneumotachograph using a corrugated metal sheet as a resistance and a metal membrane manometer as a sensing element (82). For recording intraesophageal pressures a balloon made of Vinyl plastic instead of rubber is recommended by Crane, Hamilton & Affeldt (62).

Work of breathing.—Cook *et al.* (58) discuss some of the problems and possible errors involved in calculating the work of breathing. They found that in newborn infants the work of resting breathing is 1400 gm. cm./min. (about 1 per cent of B.M.R.). In infants with respiratory distress there is an increased work of breathing and the breathing frequency increases in accordance with the principle of minimal work. Measurements of the O_2 cost of very high rates of voluntary hyperventilation have been attempted by McKerrow & Otis (141).

McIlroy & Eldridge (137) describe simplified methods for estimating the work of breathing during inspiration and some applications of these methods in various situations (136, 138, 139). Unfortunately, their basic method from which these simplifications are derived is based on an erroneous concept; viz., that the elastic work depends only on the slope of the pressure-volume curve of the lungs, whereas in fact it must depend on the absolute position of this curve as well. It is therefore difficult to judge the accuracy of their simplifications.

Surface tension effects.—Mead, Whittenberger & Radford (131) find that excised dog lungs show marked pressure-volume hysteresis and nonuniform expansion when air filled, but minimal hysteresis and uniform expansion when filled with saline to minimize surface tension effects. These authors conclude that the pressure-volume hysteresis observable during slow deep breathing *in vivo*, and which is not related to flow resistance, probably has its basis in surface forces.

Brown (41) and Clements (54) find that the surface tensions of nasal mucus, pulmonary edema fluid, and of saline extracts of lung tissue are not constant but show a marked dependence on area. By taking this into account Brown (41) revises Radford's previous estimate of the lung surface area in humans upward to 70 M^2 , a figure which agrees well with the older estimates based on histological measurements. Massion (157) suggests that the decreased pulmonary compliance which he observed following administration of curare or histamine in dogs may be related to changes in surface tension forces.

Lung volumes and maximal breathing capacity.—By subtracting "free gas" (N_2 wash-out volume) from total gas (plethysmographic method),

Bedell *et al.* (22) have estimated the volume of trapped gas in the lungs of patients with various pulmonary disorders. In one patient with pulmonary emphysema and cysts this volume was 3.67 liters.

Colville, Shugy & Ferris (57) find that the increase in end-expiratory lung volume produced by tilting in foot-down direction is proportional to the sine of the angle of trunk to horizontal. Tilting in head down direction produces no consistent change.

In the age group 40 to 88 yr. the vital capacity decreases by about 30 ml. per year according to Pemberton & Flanagan (151). The 1 sec. vital capacity is 79 per cent of the vital capacity and expressed in this fashion is independent of age, height, and weight in this age group.

Shephard (181) has re-examined sources of error in the spirometric measurement of fast vital capacity and finds advantages in the pneumotachograph. The peak capacity for voluntary ventilation as measured by the pneumotachograph is 32X the vital capacity and occurs at a breathing frequency of 90 to 100 breaths per min. [Shephard (180)], although there is little difference over the range 70 to 150.

Although the vital capacity of Japanese female divers, "Ama," is only 10 per cent greater than that of nurses and telephone operators, the maximal breathing capacity is 40 per cent greater [Tatai (188)].

Miscellaneous.—An interesting study of the mechanics of glossopharyngeal breathing (pumping air into lungs with tongue, jaws, teeth, and pharynx) in patients dependent on this form of breathing is reported by Collier, Dail & Affeldt (56). The stroke volume of this maneuver is about 60 ml. and the cycle duration 0.6 sec. Intrapulmonary pressures high enough to produce a cough having a peak flow of 290 l/min. can be developed. The somewhat similar mechanism of respiration in the frog is described by Cherian (51).

The physiology and physics of voice production is discussed by van den Berg (192). Bennett *et al.* describe an arm lift-back pressure method of artificial respiration that can be applied simultaneously to two victims (23).

PULMONARY GAS EXCHANGE

Diffusing capacity of the lung.—A standardized clinical single breath technique for measurement of the diffusing capacity of the lungs for carbon monoxide is described by Ogilvie, and his co-workers (148), who give a valuable discussion and evaluation of various factors affecting the measurement. Among these are posture, duration of breath hold, portion of expired gas sampled, intrathoracic pressure, lung volume, alveolar PO_2 , and venous COHb. The coefficient of variation for a single measurement in normal subjects was 8.5 per cent. Diffusing capacity is greatest in the supine position, least in the standing and intermediate in the sitting position. A prediction equation, derived from diffusing capacity of the lung for CO, is:

$$D_{CO} = \text{Surface Area} \times 18.85 - 6.8.$$

Bates & Pearce (20) compared the single breath and steady state CO

methods for measuring diffusing capacity and found the former to give slightly but not significantly higher results. They also obtained higher values in the supine as compared with the sitting position. They suggest a more uniform distribution of blood or an increase in pulmonary capillary volume as possible explanations for this postural effect.

Patients with mitral stenosis studied post-operatively were found by Riley *et al.* (161) to have diffusing capacities during exercise close to normal. Six patients who had been studied preoperatively showed increased values.

Lowering the body temperature of anesthetized dogs appears to reduce pulmonary diffusing capacity [Otis & Jude (149)]. Gas exchange remains adequate, however, because metabolic requirements are similarly diminished.

Three graphic methods for estimating the mean alveolar-capillary diffusion gradient for oxygen are presented by Farhi & Riley (79). A method for studying the transfer of CO_2 across the lung using $^{14}\text{CO}_2$ as a tracer is described by Bucher, Stamm & Grün (44, 98, 184).

Several papers not dealing directly with pulmonary diffusion may be of possible interest to investigators of this problem. During similar exposures to CO (concentration 0.01–0.08 per cent) some subjects absorb more CO than others for reasons not related to ventilation or oxygen consumption [Bosgeus & Friberg (33)]. The effect of posture on elimination of carbon monoxide (produced endogenously or from smoking) is described by Dahlström (66). Gibson (91) has measured the fourth velocity constant for the association between hemoglobin and carbon monoxide. The diffusion of O_2 in concentrated hemoglobin solutions has been studied by Klug, Kreuzer & Roughton (118). Allen & Root (3) find that the competition between CO and O_2 for hemoglobin is influenced by pH; the peak value of the partition equilibrium constant, M , occurs at pH 7.4.

Gas exchange.—The problem of the dead space did not end with Krogh and Haldane. Some of the factors involved are gradually becoming clarified, although the time is fast approaching when an average reader will need an annually revised lexicon at his elbow, so profuse is the growth of new terminology. Gray, Grodins & Carter (97), in an interesting theoretical analysis, distinguish between (a) anatomical dead space, ADS (from anatomical measurements), (b) virtual expired dead air, DA (Bohr equation) and (c) virtual dead space, DS (calculated from alveolar flow principles). ADS includes DS and is identical with it at zero tidal volume; both increase with increasing tidal volume, DS more than ADS. DA also increases with tidal volume and at a greater rate than DS. Experimental data obtained by these authors agreed well with theoretical expectations, especially after the exclusion of several aberrant measurements from the analysis (a procedure not foreign to many physiologists but one which causes statisticians to shudder). Among the several important conclusions reached are that alveolar ventilation increases linearly with total ventilation, and that respiratory frequency increases linearly with ventilation in exercise and in ventilation stimulated by CO_2 .

Severinghaus & Stupfel (174, 176) and Bitter & Rahn (157) have in-

vestigated the "alveolar dead space" (apparently equivalent to the parallel dead space of Pappenheimer) and its dependency on distribution of alveolar blood flow. Alveolar dead space increases in proportion to the number of alveoli which are underperfused and with tidal volume [see also Williams & Rayford (200) who find physiological dead space to increase with increasing tidal volume]. The effects of positive pressure breathing on alveolar dead space are in disagreement, Bitter & Rahn finding an increase and Severinghaus & Stupfel a decrease. Anatomical dead space increases with positive pressure breathing in both laboratories.

Asmussen & Nielsen (7) found that "physiological" dead space (Bohr equation using arterial P_{CO_2}) increased from 170 ml. at rest to 350 ml. during heavy work in which the tidal volume was 3.3 l. The effect of adding dead space is to increase the degree of uniformity of ventilation but to leave the ventilatory efficiency (N_2 wash out criterion) unchanged according to Bouhuys, Jönsson & Lundin (34). Apparently the uniformity improvement effect is cancelled out by the direct effect of the added dead space.

Dead space (Fowler's single breath N_2 technique) decreases exponentially with time of breath-hold and is also affected by magnitude of tidal volume and by rate of expiratory flow [Shepard (178)]. Dead space increases with age as does the inhomogeneity of blood flow and ventilation [Tenney & Miller (189)]. An extremely painstaking piece of work which has bearing on the dead space problem is that of Ross (164) who made detailed anatomical measurements of plastic casts (158) of dogs lungs. From this measurements and making certain assumptions Ross finds that the transit time between a given alveolus and the outside is proportional to length of the anatomical pathway. Differences in transit times from individual alveoli can lead to local differences in effective alveolar ventilation, and can account for the shape of the fast rising portion of the expired CO_2 curve. Another inference is that dead space volume will be distributed unequally among different alveoli. Although I am unable to follow all the arguments presented (e.g., the analogy between "pathway" and "inductance") this is a thought provoking paper and deserves careful study.

Alveolar-arterial gradients.—In the anesthetized dog the alveolar-arterial oxygen pressure gradient is largely due to venous admixture [Atwell, Tomaszewski & Ryan (11); Aksnes & Rahn (2)]. The gradient is higher the higher the alveolar PO_2 , and may be as much as 150 mm. when pure O_2 is breathed. The shunted blood amounts to 1.6 per cent of the total flow according to Lochner *et al* (123). [See also Elwell & Bean (77).]

The alveolar-arterial nitrogen gradient has been investigated by Canfield & Rahn (48, 157). Although the net exchange of N_2 (or other inert gas) may be zero for the lung as a whole, exchange in one direction or another will occur in individual alveoli if differences exist between the ratios of ventilation to perfusion in the individual units. The mean arterial tension of the inert gas will be greater than the mean alveolar tension (2) and if the gas has a high solubility the ventilation perfusion curve as plotted on the CO_2 - O_2 diagram will be significantly affected.

Blood gas tensions.—A rebreathing technique for estimating mixed ve-

nous CO_2 tensions is described by Collier (55). The technique is rapid and is useful in the clinical evaluation of ventilatory status, but is not sufficiently accurate for use in measuring cardiac output.

Severinghaus, Stupfel & Bradley (35, 175, 177) have made an extensive study of factors influencing the accuracy of measurement of PCO_2 and pH of blood. They point out numerous pitfalls and suggest techniques for eliminating them. Their papers should be carefully studied by all who are interested in making precise measurements of this type.

Gas exchange in hypothermia.—In the anesthetized dog (as well as in other animals) breathing ceases before circulation when body temperature is lowered. Elimination of CO_2 and uptake of oxygen in the lung appears to remain adequate however, providing artificial ventilation is maintained [Kao & Schlig (116); Otis & Jude (149); Severinghaus, Stupfel & Bradley (176)]. The physiologically optimal degree of artificial ventilation to be employed under hypothermic conditions remains an unsettled problem.

Miscellaneous.—The significance and clinical importance of the terminal rise which is sometimes observed in the single-breath O_2 test is discussed by Curtis & Rasmussen (64). Birath, Stållberg-Stenhagen & Swenson (25) point out some problems involved in bronchspirometry. For example, the better a lung ventilates the higher will be the resistance against which it has to work, because the tendency toward turbulent flow in the catheter increases. Bartlett (19) finds that "diffusion" respiration can be detected during rhythmic breathing if the frequency is slow enough, the oxygen uptake being greater in the apneustic than in the apneic phase, possibly because of a greater respiratory surface and blood volume in the more expanded lung. A new form of the alveolar gas equation is presented by Mochizuki & Bartels (134).

Hyperventilation and hypocapnia.—Hypocapnia resulting from spontaneous hyperventilation by the pilot is being suspected as a possible contributing factor in some jet plane crashes. Balke, Wells & Clark (14) have devised an ingenious method for collecting expired gas samples during actual flight and find that 78 per cent of jet pilot trainees show evidence of hyperventilation in that the calculated alveolar PCO_2 is less than 30 mm. Hg. Balke & Lillehei (15) find that performance as measured by a complex coordinating apparatus deteriorates to 85 per cent of control values at 20 to 25 mm. PCO_2 and to 70 per cent at 14 mm. PCO_2 . Hyperventilation at simulated altitude (25,000 ft.) is no more effective in depressing alveolar PCO_2 than it is at ground level [Sunahara *et al.* (186)].

Voluntary hyperventilation produces a large increase of blood flow in the nerve-blocked but not in the normal forearm [Roddie, Shepherd & Whelan (163)]. Addition of 5 per cent CO_2 to inspired gas abolishes the effect. A humoral mechanism is postulated.

CONTROL OF BREATHING

Winterstein (201, 202, 203), in a series of three articles representing the Edward K. Dunham lectures for 1955, gives an excellent summary of his

views regarding the chemical control of pulmonary ventilation with special emphasis on his "reaction" theory.

Receptors in the lungs.—Davis, Fowler & Lambert (69) have studied some responses of slowly adapting pulmonary stretch receptors, by recording action potentials from single vagal afferent fibers. Air flow was measured by a pneumotachograph (it is good to find someone using an instrument more quantitative than a pneumograph for this purpose). The receptors appear to be located in the intrapulmonary bronchi or bronchioles and their discharge frequency varies with lung volume and with inspiratory flow velocity but not with volume acceleration. During expiration, discharge ceases at a greater lung volume than it begins during inspiration. The authors suggest that the receptors generate signals related to the work of breathing.

Effects of electrical stimulation of the central stump in unilaterally vagotomized guinea pigs have been studied by Oberholzer & Steiner (147). For weak inspiratory and expiratory effects optimal impulse duration was about 2 msec.; for tonic inspiratory effects about 10 to 15 msec. The authors conclude that normal respiratory control involves relatively fast fibers (chronaxie = 0.2), whereas tonic activity involves slowly conducting fibers with high thresholds. From the results of micro-electrode recordings of single inspiratory neurones in the respiratory centers of cats, dogs and rabbits Hukuhara, Okada & Nakayama (110) conclude that vagal impulses are essentially excitatory but may become inhibitory when a sufficient number of impulses appear. They find it unnecessary to postulate more than one kind of receptor in the lung to explain the vago-respiratory reflex mechanism. From studies involving vagotomy and cooling Oberholzer & Schlegel (146) conclude that the autonomous activity of the respiratory center in guinea pigs is dependent on excitatory impulses from the lungs. Bucher & Battig (43) find that the vagal factors responsible for synchrony between pulse and breathing frequency are located almost exclusively in the left vagus.

Chemoreceptors.—Three cases in which human subjects failed to show an increased ventilation on exposure to low oxygen have been described by Brown (42). Presumably this behavior is related to some physiological anomaly of the chemoreceptors and it would be of interest to know the incidence of this lack of response in the general population. Youmans & Schopp (204) find that Cheyne-Stokes breathing occurs in dogs in which both carotid and aortic chemoreceptors have been denervated, and conclude that although these bodies may sometimes participate in this abnormal type of breathing they are not essential to it. Support for the theory that Cheyne-Stokes breathing is due to oscillation of the respiratory control system is found in the fact that this type of breathing can be induced in dogs by increasing the length of the carotid arteries with long plastic tubes [Guyton, Crowell & Moore (99)]. This procedure, which increases the circulation time from lung to brain, would be expected to decrease the damping of the natural system.

Diamond & Howe (74) have recorded afferent impulses from a branch of

the left aortic nerve in cats; and from responses obtained after induction of hypoxia and other experimental procedures, they conclude that the aortic bodies near the root of the left subclavian artery are chemoreceptors. This is in contrast to claims by previous workers that only those bodies below the aortic arch have such a function.

The effect of CO inhalation on breathing in normal and chemoreceptorless rabbits has been examined by Terzioğlu & Emiroğlu (190). In normal animals there is an immediate transient hyperpnea followed by a drop to normal; in the chemoreceptorless, there is a delayed hyperpnea followed by depression. Both groups showed an increased ventilation on return to air.

The respiratory stimulation which occurs with fall in blood pressure induced by bethanechol chloride (Urecholine) or nitroglycerine can take place in the absence of carotid and aortic chemoreceptors [Schopp, Gilfoil & Youmans (171)], but when the fall in blood pressure is prevented the stimulation of breathing is minimal or absent.

CNS mechanisms.—The recent tendency to regard the central control of breathing as having its basis in a coordinated interaction of neural activity distributed in the lower brain stem rather than in the specific activity of discrete nuclear masses or "centers" is continued in a provocative paper by Brodie & Borison (38) who propose a new terminology having a functional rather than anatomical emphasis. They suggest that the central control mechanism is made up of the following four components: oscillator, modulator, integrator and pacemaker, and present the results of experiments on decerebrate, decerebellate cats in support of their concept.

Joels & Samueloff (113) present evidence for a continued rhythmic activity of the respiratory center during apnea produced by the neuromuscular blocking agent succinyl choline. A panting type of breathing develops in decorticate cats following frontal section just anterior to the optic chiasma [Dasgupta (68)].

Effects of CO₂.—When varying concentrations of CO₂ in air are inspired during muscular exercise, Asmussen & Nielsen (8) find that the stimulus-response curve (ventilation vs alveolar PCO₂) is displaced to the left while the slope remains unaltered. When low oxygen is also present the curve is displaced still further to the left, its slope becomes steeper. The alveolar PCO₂ at which the curve begins to flatten is lower during work than at rest and still lower when low O₂ is also present. The flattening of the curves is attributed to a depressant effect of higher CO₂ concentrations on the respiratory centers.

It has frequently been suggested that the diminished respiratory response to CO₂ that occurs in emphysematous patients is due to an altered sensitivity of the respiratory center. Cherniak & Snidal (52), however, present evidence in support of a more mechanical explanation. They find that if normal subjects breathe through an artificial resistance such as to decrease the maximal breathing capacity to that of emphysematous patients, the ventilatory response to CO₂ is similar in both groups. Furthermore, the ventilatory response

to CO_2 in emphysematous patients can be increased by alleviating the respiratory obstruction by use of bronchodilators.

Effects of flow resistance.—Some effects of added resistance on ventilation in normal subjects are reported in an excellent paper by Zechman, Hall & Hull (205). One of their more interesting findings is that a resistance which brought about only a 2 mm. elevation in alveolar CO_2 at rest produced a 10 mm. elevation during exercise.

Campbell & associates (46, 47) find that expiration in both anesthetized and conscious subjects remains passive with added expiratory resistance as long as the latter corresponds to a pressure less than about 10 cm. H_2O .

Peripheral receptors.—Although Dejours, Mithoefer and associates (71, 72) find no evidence for the presence of specific ventilatory chemoreceptors in the limbs, they suggest that local chemical changes may modify the activity of proprioceptors.

Breath holding.—Craig & Cain find that breath holding time after exercise decreases with increasing grade of work, being only 6.8 sec. after the highest work rate (61). The duration of apnea in dogs following artificial hyperventilation is logarithmically related to volume ventilated, frequency, and duration of hyperventilation [Blasius, Bach & Keul (26)].

Thermal stimuli.—Increasing environmental temperature as a ventilatory stimulus in calves has been studied by Findley (80). The critical rectal temperature above which respiratory activity is affected is 40.5° . Bligh (27, 28) finds that the thermal stimulus for panting in the calf acts at the periphery, since it can be initiated in the absence of temperature rise in the blood supplying the brain.

Miscellaneous.—The increased ventilation that occurs when 100 per cent O_2 is breathed by man was measured by Baker & Hitchcock (13). Schopp, Gilfoil & Youmans (172) have examined some of the factors responsible for the respiratory stimulating effect of hemorrhage.

PULMONARY CIRCULATION

The effect of hypoxia on the pulmonary circulation still remains a problem of interest. Most investigators now appear to agree that hypoxia can cause pulmonary vasoconstriction, although there is still some disagreement regarding mechanism. Aviado, Ling & Schmidt (12) who were unable to find this effect earlier have re-investigated the problem and find that in anesthetized dogs with pulmonary innervation intact a positive effect sometimes but not always occurs. [See also Nahas (144).] Denervation of the chemoreceptors or removal of the first four thoracic sympathetic ganglia abolishes the effect completely. Their earlier failure to observe a pulmonary vasoconstrictor effect of hypoxia is attributed by Aviado, Ling & Schmidt to their experimental technique which involved direct cannulation of lobar vessels, a procedure which would largely inactivate the innervation of these vessels. The variable effects observed in the more recent experiments are attributed to the interaction of several mechanisms. Vasoconstriction tends to be produced reflexly, by hypoxic stimulation of the aortic and carotid chemo-

receptors, and locally by the action of epinephrine [see also Borst, Berglund & McGregor (32)]. On the other hand, hypoxia tends to produce active vasodilation by a direct local effect, and a passive vasodilation secondary to an increased pulmonary blood flow [see also Carlill, Duke & Jones (50)]. The net effect of hypoxia can thus be either a vasoconstriction or dilatation, depending on the relative values of these separate effects, but a vasoconstriction was observed only when the chemoreceptor reflexes were intact.

In contrast to these results are those of Duke (76) who finds pulmonary vasoconstriction in response to low oxygen in the isolated perfused lungs of cats and dogs. Duke's results are supported by previous work in other laboratories as well as in her own. Thus the problem appears not yet to be solved to everyone's satisfaction, and more experiments will have to be done.

Some effects of long term hypoxia (or strictly speaking of dwelling at high altitudes) on the pulmonary circulation are described by Hurtado & associates (165). A moderate pulmonary hypertension is present in altitude dwellers. Since this is associated with a normal or diminished cardiac output, the pulmonary vascular resistance must be increased, although how much of this is a reflection of the increased viscosity of the polycythemic blood is not evaluated.

Nahas (143) studied the effects of hypoxia on the pulmonary circulation in dogs during apnea in order to eliminate effects that might arise secondarily from changes in ventilation and observed an increased pulmonary vascular resistance [see also (144)]. In Cournand's laboratory, acetyl choline has been found to cause pulmonary vasodilation in man especially after vasoconstriction has been produced by hypoxia (102), but Borst, Berglund & McGregor (32) found less consistent effects in dogs. Serotonin is a potent vasoconstrictor of the pulmonary vessels producing a five-fold increase in pulmonary vascular resistance [Rudolph & Paul (166)]. Heiman *et al.* (105) have examined some respiratory factors affecting pulmonary blood flow, and suggest that pulmonary vascular resistance tends to increase during inspiration especially at large lung volumes. "Pulmonary" blood volume as measured by Rapaport *et al.* (159) in patients with mitral stenosis shows an inverse logarithmic relationship to pulmonary vascular resistance but a direct linear relationship to cardiac output. "Pulmonary" hematocrit was calculated to be 0.92 to 0.97 of large vessel hematocrit, and the conclusion was drawn that pulmonary capillary hematocrit was less than 0.75 of the large vessel hematocrit because the pulmonary capillary volume is probably no more than 10 per cent of the total measured "pulmonary" volume. On the other hand, Lilienfield *et al.* (120) concluded that the "pulmonary" hematocrit is not significantly different from large vessel hematocrit and that axial streaming does not occur in pulmonary capillaries to any significant extent.

HYPOXIA

Hypoxia is still a poorly understood condition. Other than saying it is a situation in which less than the optimal amount of oxygen is present, we have no adequate definition of it. Although we have a large amount of information

regarding its effects on various physiological functions, we know much less about the basic alterations in cellular mechanisms that occur when it exists. Profound physiological effects can be observed, even though the rate of oxygen uptake by the body may remain undiminished, at least within the precision of our measurements. Indeed it appears in many instances that oxygen consumption falls only when the situation approaches irreversibility.

Pugh (155) reports some observations made on Mount Everest during the successful British expedition of 1952-53. Of interest is the fact that the barometric pressures as actually measured in the mountains were higher than those read for the corresponding altitudes from standard tables. Above 18,000 ft. the differences were physiologically significant. For example, a pressure of 350 mm. according to the tables corresponds to 20,000 ft. but on the mountain this pressure occurred at 22,000 ft. Although no actual measurement was made at the summit (29,000 ft.) Pugh estimates that the pressure there was close to 250 mm. in contrast to 236 mm. as predicted from the tables. It can be estimated by extrapolation that one could go to 34,000 ft. on a mountain before encountering a pressure as low as 236 mm. These observations should be of some interest to investigators dealing with equivalent altitudes in decompression chamber work.

Among the physiological observations made by Pugh are the following: Resting ventilation rose on the average from 7.3 l./min. at sea level to 15.8 l./min. at altitudes of 18,000 to 25,000 ft. At 24,000 ft. ($P_B = 308$ mm.) alveolar PCO_2 was 16.8 mm. and PO_2 was 34.1. Supplementary O_2 in the inspired air at altitudes of 18,000 ft. and above produced a small immediate rise in alveolar PCO_2 followed by a slower gradual rise over many hours. On removal of O_2 the alveolar PCO_2 quickly returned to its original value.

Measurements of ventilation, oxygen consumption, hemoglobin, rectal temperature, pulse rate, and blood pressure made during the 1955 Frankfurter Himalaya Expedition are reported by Brendel (36).

Chiodi (53) has studied respiratory responses of both newcomers (2 to 54 days) and long term residents at altitudes of 3990 and 4515 m. in the Andes. Contrary to some previous impressions, newcomers at altitude appear to have a higher resting ventilation and to show a greater response of CO_2 in the inspired air than do the long term residents. The breathing of oxygen depresses the ventilation of newcomers more than that of long term residents. According to Chiodi resting ventilation reaches its peak value after about three weeks at these altitudes and at some later date must fall to a lower level, although it always remains well above sea level values.

Hurtado & associates have reported some of their studies on the native residents of Morococha, Peru, who live at an altitude of 14,900 ft. (111). These people have an alveolar PO_2 of about 50 mm. Hg and an arterial saturation of about 80 per cent yet are able to perform remarkable amounts of physical exercise, and more efficiently than can sea level dwellers. Whether this increased efficiency is to be explained by physiological readjustments or on the basis of altered cellular metabolism is still an open question. The ex-

tensive data reported will be welcomed by all who are interested in problems of adaptation to hypoxia.

A comparison of respiratory adaptations to altitude hypoxia with those to hypoxia of circulatory origin is made by Husson & Otis (112). Barker (18) reports on an increased affinity of hemoglobin for O_2 which develops in various mammals during acclimatization to hypoxia. She attributes much significance to this as a determining factor in tolerance to hypoxia and finds the importance of increased amount of hemoglobin to be minimal. Pace, Meyer & Vaughan (150) find that the decrease in hemoglobin which occurs when acclimatized individuals return to sea level is the result of both decreased erythropoiesis and increased erytholysis. About two-fifths of the increase in circulating red cells occurring in rats during exposure to a simulated altitude of 15,000 ft. for 30 to 60 days can come from the spleen, and about three-fifths from new production [Cook & Alafi (59)]. Large increases in the myoglobin content of rats living continuously at an altitude of 12,500 ft. are reported by Vaughan & Pace (195). Physiological and psychomotor changes occurring in man during acute exposure to 20,000 ft. simulated altitude are described by Shephard (179).

Born, Dawes & Mott (30, 31) report further on the physiology of fetal and newborn lambs. Reduction of the inspired PO_2 of the mother lowers the HbO_2 saturation of both mother and fetus in a parallel fashion. Fetal lambs close to term respond to the anoxemia so produced by an immediate increase in heart rate, blood pressure, umbilical blood flow and O_2 capacity of the blood. In immature fetuses (< 72 days gestation age), such responses were minimal or absent. The results are believed to be related to the state of development of the autonomic nervous system. The O_2 consumption of fetal and newborn lambs decreases if the HbO_2 drops below 35 per cent (1).

Flückinger (83) finds that the O_2 consumption of rats exposed to 10 per cent O_2 or to air at 350 mm. pressure is decreased by 30 per cent within 15 minutes, while the rectal temperature decreases only slowly. He concludes that reduced heat production is a primary effect of hypoxia, and that body temperature drops secondarily. During a 14-day exposure rectal temperature returned to normal in three to four days, while oxygen consumption more gradually approached the control values. Ullrick *et al.* (191) found no change in total metabolic rate or rectal temperature during an 11-week exposure of mice to 18,000 ft. simulated altitude although no measurements were made during the first week of exposure. The oxygen consumption of various tissues was measured at the end of exposure and except for adrenal (increase) and kidney (decrease) no changes were found.

Björk, Johansson & Schmid (24) find that hibernating hedgehogs can tolerate exposure to pure N_2 for 1 to 2 hr., but in the nonhibernating state only 3 to 5 min. Unfortunately, no control experiments were done with both groups at the same body temperature.

Moderate hypoxia enhances, but more extreme hypoxia inhibits, audiogenic seizures in rats [Mitchell & Hitchcock (133)]. In anesthetized cats

hypoxia decreases or prevents shivering [Hemingway & Birzis (106)]. Hypoxia (*in situ*) at first increases and later decreases the respiration of mouse diaphragm (measured *in vitro*). The *in vitro* water content is increased [Hannon & Cook (101)].

The critical HbO_2 saturation of coronary venous blood is about 6 or 7 per cent, corresponding to an O_2 partial pressure of 4 or 5 mm. Below this value glycolysis occurs and the heart fails [Bretschneider *et al.* (37)].

McNeil (142) finds that during the reactive hyperemia following a 5 minute occlusion of the forearm, the percentage of HbO_2 in the effluent venous blood rises above the resting value and concludes that hypoxia is not an important factor in maintaining the increased flow.

Evidence for the existence of a humoral erythropoietic factor in the blood of animals which have been exposed to hypoxia is accumulating [Stohlgman & Brecher (185); Prentice & Mirand (154)]. Such a substance has also been detected in nonprotein plasma extracts from humans with polycythemia vera and with secondary polycythemia [Linman & Bethell (122)].

PULMONARY EDEMA

Aravanis *et al.* (6) have studied pulmonary edema produced by stimulation of the central nervous system with various agents (veratrine injection, electrical stimulation, injection of air or saline), and suggest that vasomotor activity of the pulmonary vessels may be involved. Cassen, Gutfreund & Moody (49) find that severing the spinal cord in rats prevents the induction of pulmonary edema by epinephrine. Dogs with experimental aortic insufficiency develop pulmonary edema when the renal arteries are constricted [Hawthorne, Brownlee & Jason (103)].

MISCELLANEOUS

The following listings include many papers which deserve a better fate: Studies of underwater swimming (93, 94, 95, 183); Effects of oxygen under high pressure (21, 115, 152, 153); Effects of pressure breathing (117, 125); CO_2 and acid base balance (17, 63, 70, 88, 114, 132, 162); Oxygen uptake and utilization (4, 87, 107, 108, 126, 182, 194); Oxygen secretion in fish (168, 170); Water and electrolyte content of the lung (161, 5, 81); Ciliary activity in the trachea (65); Broncho-constriction by cigarette smoke (124); Equilibrium between cytochrome oxidase and CO (196); Reactions of myoglobin with gases (90); Combination of nitric oxide and hemoglobin (92); Oxygen tension in subcutaneous gas pockets (157, 193); Emulsion reversal by inert gases (173); Textbook on pulmonary emphysema (16).

Techniques and apparatus.—Gas analysis: CO_2 (96, 198); N_2 (199); CO (89, 121); total gas in blood (157); oximeter (76); (A-V) O_2 recorder (100); O_2 in fish blood (169); O_2 consumption of animals (73); artificial breathing in small animals (119); oxygen mask (187); breathing valve (140); Ventilation meter (Venturi principle) (197); self regulating artificial respirator (85).

This review has been selective rather than exhaustive of literature that

has appeared during the year ending in June, 1957. The basis for selection has depended on what I have happened to read that interested me rather than on any logical system. This rather haphazard approach has doubtless led to the omission of numerous worthy papers, and to the authors of these, as well as to the readers of this review, I offer my apologies.

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THE PHYSIOLOGY OF THE SKIN^{1,2}

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The richest development in cutaneous physiology has been the appearance of Stephen Rothman's scholarly, comprehensive, and exhaustive treatise on every aspect of the physiology of the skin. With the aid of six experienced investigators, he has reviewed the entire world literature in this field (1). His treatise will be a constant inspiration and invaluable assistant to all who would study the skin. A second signal contribution has been a monograph by Montagna (2) on the structure and function of skin. Montagna's book focuses on the histology and histochemistry of the skin but in many sections cellular physiology is reviewed in detail. Finally a short survey of the physiology of the skin with reference to dermatology has appeared (3). Excellent regular annual reviews of research in the field have also been published (4 to 10).

Even a cursory perusal of Rothman or Montagna's works gives one an awareness of the diversity of skin. The physiology of skin directly embraces over half of the other reviews in this volume. Yet the unique specialization of this outer sheath of life has led to its separate coverage in this chapter.

In reviewing the advances we would like to return you to the anatomical elements of skin. Accordingly we have divided the material into nine sections in which we have attempted to focus down on unifying conceptual advances, rather than the striking yet solitary new fact. Furthermore, findings on mammalian skin will be emphasized.

KERATINOCYTE

Mammalian skin is basically divisible into epidermis and dermis. The epidermis is a binary system of two cell lineages (11): the keratinocytes (3) which form keratin and the melanocytes which form melanin. In 1953 the general epidermal functions were carefully reviewed (12). The physiology of the keratinocyte is basically the story of the holocrine secretion of a fibrous protein (13). Such key cellular studies are progressing, but as yet an integrated over-all picture cannot be obtained. Nevertheless in the past six years significant avenues have been explored with reference to the keratinocyte itself.

The first of these is a demonstration that epidermal cells may be grouped in a fashion analogous to erythrocytes. A number of reports in the literature

¹ The survey of literature extends from June 1, 1951 to June 1, 1957.

² The following abbreviations are used in this chapter: DOCA (deoxycorticosterone acetate), MSH (melanocyte-stimulating-hormone), dopa (3,4-dihydroxy-phenylalanine).

attested to the fact that epidermal cells showed no agglutinating phenomena when mixed with various blood sera. However, in 1956 a group at Cambridge (14) reported an ingenious demonstration that human epidermal cells (keratinocytes) did indeed possess A and B blood group antigens. Their technique is labelled a mixed agglutination reaction since simple agglutination does not occur. Actually, in practice, dispersed epidermal cells are exposed to strongly titred blood-typing sera. Following this the cells are washed, and then exposed to suspensions of various types of erythrocytes. In the presence of a sequential system of epidermis from a person with A group blood, anti-A grouping serum, and group A red blood cells, one sees grouping of the red cells on the surface of the epidermal cells. The red cell is thus an indicator under phase microscopy of the adherence of anti-A agglutinin on the epidermal cell surface. The red cell and the keratinocyte share the same antibody, therefore showing that at least two receptors exist which are capable of joining with the antigen.

This fascinating discovery has been confirmed by another group (15) who show that A and B antibodies are absorbed selectively on epidermal cells. These immune responses are of significance in considering why heterotransplants fail to take. However, other immune reactions are also operative since transplants of A epidermis to A recipients have not led to successful grafts. The Cambridge group has continued its studies to more precisely characterize the keratinocyte immunologically, but early efforts (16) have failed to demonstrate any antigens to the following anti-sera: Rh, MN, Lewis, Lutheran, and P. Presumably these anti-sera are of insufficient potency since high titres are needed. In any event it is now possible to state that the human epidermal cells possess the same grouping as their colleague erythrocytes.

A second major area of study has been concerned with glycogen in the epidermis (17 to 24). Normal epidermis shows little or no glycogen, yet it is becoming increasingly apparent that under certain circumstances glycogen may appear in rather large amounts. The most striking example is the finding of Claude Bernard nearly a hundred years ago that fetal epidermis has abundant glycogen (25). The epithelium of the mucous membrane is also rich in glycogen. Furthermore, it is present in the outer root sheath of active hair follicles. However, the most significant finding has been the study of the effect of injury on the epidermis. Lobitz & Holyoke (21) have made a detailed study of glycogen response in normal human epidermis following mild injury produced by stripping off the stratum corneum with Scotch tape. They have found a rather precise and predictable sequence of events. Within 5 min. the basal cells of the epidermis had swollen to twice their original size. By 8 hr. the basal keratinocytes were all heavily laden with glycogen. At 48 hr. the entire epidermis was again free of glycogen, but by 72 hr. a second wave of glycogen accumulation was seen in the keratinocytes of the mid-epidermis. This second wave disappeared within the week. It is evident that a heavy load of glycogen in the basal cells is a true indication of injury to the epidermis during the preceding 8 to 24 hr. Likewise glycogen in the mid-

epidermis also indicates an injury of from 3 to 5 days prior. Other workers have shown this same glycogen accumulation in skin grafts and in regenerating epidermis. Montagna's group (24) postulates that the presence of glycogen reflects a slowing up of metabolic processes. When cells are actively dividing or rapidly keratinizing, glycogen may be used up rather than stored. They feel injured cells, neither dividing nor undergoing complete keratinization, are another example where one would expect glycogen accumulation. Fetal epidermal cells are viewed as sluggish and as showing incomplete keratinization. Accordingly glycogen accumulates. It is interesting that the eccrine sweat gland (26) shows glycogen in the resting cellular state, but that this disappears following secretory activity. Moreover, the apocrine and sebaceous gland secretory cells which are in continuous secretory activity are devoid of glycogen. All of these findings support the view that glycogen may be viewed as an index of the activity of the keratinocyte.

Another area of advance has been in the study of the effect of vitamin A on the keratinocytes (27 to 33). Fell & Mellanby have found that cornification of the ectoderm of the chick embryo can be completely suppressed by a high level of vitamin A. They prepared explants of skin from a seven-day-old embryo. Within seven days these explants developed into thick keratinized squamous epithelium when grown *in vitro* in a normal medium. In the presence of 1000 I.U. of vitamin A per 100 ml. of medium a dramatic remarkable effect was found. Here the ectoderm differentiated into mucous-secreting ciliated mucosa. A totally different epithelium had arisen. Vitamin A had transformed the keratinocyte into a mucous cell. Next Fell & Mellanby demonstrated that transfer of the mucus explant back to a normal medium led to a reversal of the metaplasia. In the normal medium the skin again became skin and lost its mucosal property. It now appeared as a stratified keratinizing membrane. These tissue culture studies document the signal importance of vitamin A in the development of the keratinocyte. Weiss & James (31) have confirmed this by showing that single chick embryo ectodermal cells may develop into cuboid mucous cells after a brief 15 min. exposure to 0.06 per cent vitamin A. A recent review of the clinical effects of vitamin A has appeared (32). Topical and systemic administration were without demonstrable histologic effects on the epidermis of normal adult subjects.

Finally, electron microscopy (34 to 37) has provided illumination on the problem of how the keratinocytes attach to one another as well as to the underlying collagen of the corium. Since any separation in these moorings may be manifest as vesicle or bulla, the problem deserves attention. Selby (37) has reported in detail on the problem in human and rodent skin. She has found that the intercellular bridges, long assumed to represent filaments passing between the epidermal cells, are an artifact of light microscopy. No filaments pass between the cells. Rather does the bridge represent intracellular filament mooring granules which occur at matching points on two adjacent cells. These bridges are adherence points since elsewhere the cell membranes are separated by an intercellular space. At the dermo-epidermal

junction there is a submicroscopic membrane. Again no epidermal or dermal mooring filaments are seen to cross this dermal membrane. The basal cell membrane appears bound to the dermal membrane by an amorphous material found in a 300 Å space between cell and membrane. Selby's work has clearly dispelled the former view that keratinocytes are anchored to the dermis and to each other by filamentous processes. It now appears that an intercellular cement substance is responsible.

MELANOCYTE

The basic biochemistry of the formation of melanin (38, 39) developed by Raper and his co-workers has stood the test of time. Much of the work of the past three decades has been concerned on the one hand with the elaboration of the chemistry of the tyrosine-tyrosinase-melanin system, and on the other with the demonstration of the presence of this laboratory system in living mammalian tissue. Two recent advances of the latter type are of particular interest. 3,4-Dihydroxy-phenylalanine, which became famous with Bloch's discovery of the dopa reaction, and had been shown to be the first product of the action of tyrosinase on tryosine, had never been demonstrated in normal mammalian skin. Foster & Brown (40) were able to establish its presence by paper chromatography in dialysates from tyrosine-active normal pigmented mouse skin incubated with and without the addition of L-tyrosine. Dopa was not identified in dialysates from albino mouse skin in which the tyrosinase system is absent. A second such development concerns the clarification of the position of phenylalanine in the tyrosine-tyrosinase system. Phenylpyruvic oligophrenia, a recessive metabolic disease in which the hydroxylation of phenylalanine to tyrosine cannot occur, is characterized by high blood levels of phenylalanine and low blood levels of tyrosine. The clinical observation that these patients have light pigmentation and poor sun-tanning ability had suggested that tyrosine deficiency was the cause of this hypopigmentation (41). However, when these patients are maintained on a diet low in phenylalanine they show pigment darkening (42). This new fact suggested that rather than tyrosine deficiency, an inhibitory action by phenylalanine itself might be the cause of hypopigmentation in these treated patients. Miyamoto & Fitzpatrick (43), using tyrosinase prepared from the Harding-Passy melanoma, were able to confirm L-phenylalanine's inhibitory action on the tyrosine-tyrosinase system. Since the concentration at which they found inhibition to begin corresponds to the upper normal blood concentration of phenylalanine, they propose that it be considered as one of the factors controlling normal melanin pigmentation.

The biochemical control of checks and balances surrounding the pigimentary system in the mature organism has recently been shown to be equally important in the embryonic state. Using the ectoderm of the urodele (*Ambystoma maculatum*) embryo, Wilde (44) performed inhibition and facilitation experiments in tissue culture by controlling the medium content of tyrosine,

phenylalanine, and structural analogues of these. He found that the type and degree of differentiation of pigment cells of the neural crest were dependent on the metabolites present in the medium.

The melanocyte-stimulating hormone (MSH) of the pituitary gland (intermedin, melanophore dilating principle, melanophore hormone) had been studied extensively in fish and amphibia, but not in man. Stress situations such as pregnancy and infectious disease were known to cause hyperpigmentation in man. Addison's disease was well known for producing the same phenomenon. Cortisone and hydrocortisone (45), which have some inhibitory effects on the pituitary, were demonstrated to lessen the hyperpigmentation of Addison's disease, and hypopigmentation was also known to be present in pan-hypopituitarism. Lerner, Shizume *et al.* (46, 47) administered MSH for the first time to human subjects. They found that MSH causes darkening of human skin similar to that seen in Addison's disease. New dark spots and new nevi are formed. This hyperpigmentation may be visible as soon as 24 hr. after the administration of a single large dose. In all cases the pigmentation returned to normal a few weeks after cessation of the drug. Blood and urine values for MSH were found to rise and fall simultaneously. No sex difference in these levels was found in normals, but MSH levels were found to rise from the second month of pregnancy to term. They demonstrated that norepinephrine and epinephrine are able to block the effects of MSH on the melanocyte by local action, while cortisone and hydrocortisone were shown not to have a local or direct inhibitory effect on the melanocyte.

That ACTH can produce Addison-like pigmentation and darkening of nevi in human skin, and that clinical grades of ACTH are highly effective in causing hyperpigmentation in frog skin has aroused interest in the relationship of ACTH to MSH. This apparent overlap of the physiological effects of MSH by ACTH has been clarified by several new developments. Lerner *et al.* (46) found that in a human subject a single injection of ACTH containing a known amount of MSH caused the same increase in urinary MSH excretion as did a single injection of that amount of MSH alone. Using frogs they found that 2 U. of MSH with a trace of ACTH caused pigment darkening, but that 0.5 U. of MSH, together with 400 times the ACTH in the first preparation, did not cause pigment darkening. They concluded from these physiological observations that ACTH does not increase production of MSH by the intermediate lobe. From the chemical standpoint preparations of MSH (48 to 51) have been purified to the extent that the most potent preparation now has a strength of 1 to 2×10^{10} U./gm. It is composed of two polypeptide fractions (α and β MSH), having molecular weights of about 4000, and containing about 15 different amino acids. The chemical and physical properties of the fractions are different from each other (and from those of ACTH); 0.5 mgm. of these preparations tested by the ascorbic acid depletion method showed no ACTH activity. Vasopressor activity was also

absent. Tests in the same laboratory of two highly purified ACTH fractions showed them to have only about one per cent MSH activity, probably representing contamination. At present the evidence favors the view that ACTH and MSH are not only chemically but also physiologically independent.

The relationship of the dendritic cells of the basal cell layer of the epidermis (melanocytes) to the dendritic cells in the epidermis above the basal layer ("high level" dendritic cells, Langerhans cells) has been the subject of great divergence of opinion. While the melanocyte has long been identified as the dopa-positive melanin-producing unit of skin, the high level cells have been demonstrated to be dopa-negative. Melanocytes have been observed in mitosis while high level cells have not. The function of the high level cells has been variously ascribed. They have even been considered as an integral part of the sensory receptor system, as intraepidermal pain receptors. Masson (52), using gold chloride preparations, considered the Langerhans cells to be effete desquamating melanocytes. This proposition has been investigated in an ingenious and logically convincing series of experiments by Billingham & Medawar (53). They first demonstrate that the dendritic cells in the basal layer, visible with out special treatment, dopa-positive, staining supravitaly with quinone-imine dyes, and the clear cells are all "preparation images" of the melanocyte. They then show that the high level cells, nonpigmented, dopa-negative, never seen in mitosis, staining with metallic gold impregnation techniques and with supravital quinone-imine dyes are also "preparation images" of the one high level cell. They then demonstrate that both these classes of cells are morphologically identical and that they not only occur in a one to one ratio to each other (guinea pig ear), but that high level dendritic cells are present only in those areas of the skin where melanocytes are present, i.e., they are both absent from the tongue and cornea of the guinea pig. On guinea pig trunk skin, melanocytes are present in the "hills" (epidermal ridges) but absent in the "valleys" (spaces between ridges), while the high level cells are distributed over both areas. They found, by stimulating mitosis with colchicine injections, that the hills represented the only area of mitosis and acted simply as a supply center of dendritic cells which then spread upward and out over the valley areas from these active centers. The argument that regeneration of dendritic cells might occur from the corium was disposed of by an experiment (54) in which pure epidermal cell suspensions from pigmented ears (rabbits) were seeded onto unpigmented areas from which the epidermis had been entirely removed. The regenerating epithelium (both keratinocytes and dendritic cells) was pigmented, i.e. followed the donor (pigmented epidermis) and not the receptor (unpigmented corium) site, showing that epidermal cells are reproductively self-sufficient. They thus convincingly proposed the identity of the melanocyte and the high level dendritic cell, the latter representing, in their view, merely the older, enzymatically inactive melanocyte, no longer capable of mitotic activity and on its way to eventual desquamation. In this sense, the melanocyte must now be considered to be a squamous cell.

HAIR

Among the many interesting properties of the hair follicle, the cyclic nature of its growth is unique (55). The cycle differs in time and distribution with the species studied. In many mammals it is seasonal. In the mouse and rat, growth cycles are frequent and precede posteriorly and dorsally in waves. In man and the guinea pig, growth is neither seasonal nor wave-like. However, within these pattern differences, the phases of growth and rest of the individual hair are much the same regardless of species. Since it is possible to histologically identify the position of a given hair relative to its own growth cycle, the histochemistry of the hair follicle can be studied, alone among normal tissues, with absolute knowledge of the type of activity of the tissue at the time of study. Many of the recent advances are concerned with this type of correlation. Thus, only during the period of hair proliferation are acid mucopolysaccharides (56) present in the dermal papilla. Cytochrome oxidase activity centers in the matrix (57). Esterase (58) and dehydrogenase (59) activity are most prominent in the dermal papilla and the outer sheath during the growth phases. The demonstration of S-H groups (60) is strongest at the zone just above the bulb where hair production actually occurs and they correspond quantitatively to the keratinization activity during hair growth. That this cyclic activity is not confined to the hair alone was demonstrated in mice by Chase *et al.* (61). In early anagen (growing phase) they found the epidermis became two to three times its resting thickness. However, at mid-anagen the epidermis abruptly returns to its former thickness while the corium at this time thickens by half and the adipose layer by two to three times. The corium and the adipose layer return to normal with the end of anagen. It has long been established that the capillary bed surrounding the hair follicle increases during the growth phase from a slight vestigial net to a dense rete. The fragility of the capillaries of rat skin has recently been found to correlate with the hair cycle (62). Fragility is lowest during the resting phase and highest just prior to the growth phase.

The fascinating endeavor to "grow hair" has continued to interest scientists as well as laymen. These efforts should be classified in several ways: by whether the experimental subject is man or a laboratory animal, by whether the agents used act pharmacologically or simply as irritants, and by whether a growing hair is simply induced to grow more rapidly or a new growth cycle is induced in a resting hair. The understanding of recent developments is clarified when these criteria are kept in mind. It has long been known that "any nonspecific irritation, short of damaging the hair follicles, may activate a new hair cycle in animals" (63). Hyperemia has been viewed as the common denominator in this phenomenon which may result from agents as diverse as ultraviolet light, freezing, or extract of mistletoe. While this type of induction of a new hair cycle is an established fact in some experimental animals, the observations of Ressman & Butterworth (64) indicate that irritation affects the human hair follicle quite differently. In an institution for the feeble-minded, they studied 53 inmates in whom circumscribed

cutaneous areas of the upper extremity were subject to frequent habit sucking and biting. They found that in addition to hyperkeratosis and hyperpigmentation an excessive growth of hair occurred in 13 (25 per cent) of these patients at the traumatized sites. The length of individual hairs and the total number of hairs were both increased in comparison with control areas on the same subjects. From histological studies they found no evidence of the formation of new follicles or the activation of resting follicles. However, delayed shedding of old hairs was frequently noted so that one follicle might contain several hairs. Thus, the effect (when it does occur) of irritation on the human hair follicle is to stimulate the growth of growing hairs and deter shedding but not to activate resting follicles. Much work has been done recently on the effect of hormones on hair growth. Here, again, species difference is the key to interpreting the physiology. The work of Mohn (65) and Houssay (66) with rats and mice may be taken as exemplifying recent work with hormones in animals. Gonadectomy was found to increase the hair growth rate in certain strains of mice but not in rats. Adrenalectomy increased the rate of hair growth in both rats and mice but did not affect the duration of the hair cycle. High (nonphysiologic) doses of cortisone and hydrocortisone inhibited hair growth in both species when given topically or parenterally. Androgen and estrogen inhibited hair growth in both species, but the threshold to estradiol benzoate was found to be 125 times lower for mice than rats. ACTH inhibited hair growth in rats. Contrastingly, in human subjects parenteral cortisone and ACTH have been found to cause the growth of hair in patients with alopecia areata ("idiopathic" baldness) (67, 68, 69). Unfortunately, this growth, when it does occur (16 of 22 patients in one series), tends to be irregular, with hair of the lanugo type, and is impermanent unless the drug is continued above a rather high critical level (100 mg. of cortisone daily). DOCA has been shown to have no effect on hair growth in human subjects. Thus, when these observations in human and animal subjects are considered as a whole, the growth reaction to irritants and pharmacological agents is seen to depend on a species specific response of the hair follicle itself.

Another recent advance concerns the problem of the regeneration of hair follicles in wounds. It had been generally thought that when a wound, from which the skin had been extirpated to such a depth that all of the follicles are removed, is re-epithelialized no new follicles were formed, i.e. epidermis regenerated by extension from the edges, but the destroyed follicles were not replaced. Lacassagne & Latarjet (70) challenged this view when they reported the redifferentiation of hair follicles in mouse skin in scars following ultraviolet light burns. This observation has recently been confirmed by two other workers. Breedis (71) has studied this problem thoroughly in rabbits. He removed 2.5 cm. discs of skin from their backs to a depth including the fatty layer. Special precautions were taken to prevent contraction (he fitted a metal ring with projecting legs into the edges of the wound) injury (a glass cover over the wound) and drying (special dressings). The area healed by

formation of a scab and granulation tissue. With this technique he found that, as the wound healed, thickened ridges of epithelium were formed in concentric circles from the outer edge. The ridges nearest the periphery began to show a line of budding new follicles about 30 days after operation. At the same time and site the earliest formation of new dermal papillae could be seen. These follicle buds and dermal papillae went on to form new mature hair follicles with sebaceous glands. This process of hair follicle formation is exactly the same as that occurring in the rabbit embryo except for the formation of epidermal ridges. It is of particular interest that the dermal papilla can also be formed in an area where the corium has been entirely removed; for it has been established that while the epidermal bud may be formed in tissue culture or hairless mice, the presence of the dermal papilla is essential for the development of a normal functioning follicle. Breedis' work has been confirmed (72) by the observation of the same process of follicle regeneration in an x-ray wound on the back of a rabbit. While these observations have not been made in man, it is now certain that in the mouse and rabbit hair follicles can be formed *de novo* in repairing wounds.

SEBACEOUS GLAND

The sebaceous gland has continued to merit and to receive considerable attention. A major area of endeavor has been the unravelling of the endocrine factors associated with sebaceous gland growth. Ebling (73 to 76) has continued his fundamental studies which now indicate that in rats testosterone increases both cell proliferation and cell size, thus accounting for the sebaceous gland enlargement known to follow androgens. Estradiol, in contrast, reduces the gland size by accelerating cell breakdown. It does not inhibit cell proliferation nor reduce cell size. Interference with the adrenal-pituitary system is followed by an enlargement of the sebaceous glands due to a delay in breakdown of sebaceous cells. Also Rothman's group (77, 78, 79) has been active in studying hormonal effects. They have suggested the possibility that the pituitary gland in rats has a sebaceous gland trophic factor. However, all of the evidence is indirect and involves an elaborate form of reasoning. In any event it must be emphasized that none of the data obtained can be directly transferred to the study of the sebaceous gland in man. Species differences abound here as elsewhere and, more important, there has been a failure to recognize that the dosage levels given the experimental animals are far in excess of any levels to be found in, or given to man. Shelley *et al.* (80, 81) have shown that topical, systemic, and locally implanted androgens and estrogens, as well as other hormones, are without any significant effect on the sebaceous gland in normal adult male subjects. Indeed, none of the cutaneous structures showed histologic change. The hormonal relationships are of vital importance in understanding the growth patterns of the sebaceous gland and undoubtedly the next years will see more research in this field.

For many years the sebaceous gland has been viewed as a holocrine organ whose secretory rate and function were under the control of the lipide film on

the skin surface (1). The classical view, supported by many workers, has been that sebum delivery occurs immediately upon removal of the lipoids from the skin surface. This process is at first rapid, but progressively slows down as more and more sebum appears on the surface, finally reaching a standstill when the lipide level reaches an equilibril point. Kligman & Shelley (82) have published an extensive experimental study of the physiology of the sebaceous gland in man. Their findings indicate that the gland is not under the control of the level of surface lipide. Rather, the gland was found to secrete and deliver sebum at a continuous rate, characteristic of the subject and the area studied. The major quantity of sebum of the forehead is produced by a unique type of pilosebaceous unit which is essentially a huge gland with a rudimentary hair. This unit has a relatively large reservoir of preformed sebum which can be mechanically expressed as a clear colorless drop of oil. Previous investigators failed to recognize the wicking effect of the stratum corneum in the presence of this reservoir. In this way, removal of the surface lipide is followed by rapid replacement but the replacement in no way reflects sebaceous secretion. Furthermore, it was found that quantitative determination of sebum levels demands complete immobilization of the subject as well as isolation and protection of the test site. No data were obtained to support the recently advanced concept that sweating promotes the delivery of sebum (83, 84). Nor was there any evidence that emotions have an immediate effect on sebum delivery as had been found by several groups (85, 86). Finally, the muscles of piloerection were shown incapable of propelling sebum to the surface. Other workers had also shown that cutis anserina had no effect on sebaceous gland function (87).

The possibility that sebum might arise from sources other than the sebaceous gland was carefully explored by Hodgson-Jones (88). He found no evidence to support such a view which had been proposed in isolated reports. Finally, Herrmann, Prose & Sulzberger demonstrated (84) very convincingly that sweat will leach out the endogenous lipides of the stratum corneum. Such a mixture of lipide and sweat may readily be confused with sebum.

APOCRINE SWEAT GLAND

Aoki (89, 90) has reported his extensive investigations of the apocrine sweat glands to be found on the hairy skin of dogs. Forty-five dogs were studied from the histologic as well as physiologic aspect. Spontaneous sweating in response to a hot environment or excitement was not seen, except in rare instances. However, the glands were found to be highly reactive to intradermal epinephrine, norepinephrine, acetylcholine, methacholine, and pilocarpine. Threshold concentrations varied from 10^{-3} to 10^{-7} , and the gland responded for 10 to 90 minutes. The sudorific effect of epinephrine was abolished by dihydroergotamine and that of acetylcholine, mecholyl, and pilocarpine by atropine. Sympathectomy did not affect the sensitivity of the sweat glands. Axon reflex sweating from nictotine could not be demonstrated except in a few areas in some dogs. Significantly it was in these areas that

spontaneous sweating had been noted. Direct radiant heat proved to be an effective stimulus to the sweat glands generally. However, following such a stimulus or epinephrine or norepinephrine injections, and to a lesser extent acetylcholine or methacholine, the sweat glands locally remained refractory to a second stimulus. Histologically, Aoki could demonstrate that these apocrine glands showed striking specific morphologic changes during secretion. After stimulation nearly all of the secretory cells became columnar, protruding a cytoplasmic knob into the lumen, the nuclei became elliptical and the myoepithelial sheath showed a remarkable thickening. The active and inactive gland could be sharply distinguished on purely histologic grounds. Finally, it was concluded that the sweat glands in the dog are lacking in innervation since spontaneous sweating could not be elicited in the presence of a functional gland.

Lovatt Evans and his group (91 to 94) have brought forth a most illuminating series of papers on the physiology of sweating in horses. The sweat gland in the horse is apocrine in type, responding to circulating epinephrine but not to neural influences. 1.0 μ g. of epinephrine per kilogram given intravenously will produce generalized sweating. Intradermally as little as 0.1 μ g. of epinephrine will produce local sweating for over an hour. This local area will then remain anhidrotic for several days. Indeed, general anhidrosis may be precipitated by intravenous epinephrine. The epinephrine antagonists were without effect on the epinephrine response. Norepinephrine is without effect on the horse's sweat gland, despite the fact that it produces piloerection whereas epinephrine does not. Acetylcholine either intravenously or intradermally produces sweating. Evans postulates that the acetylcholine acts indirectly, by vasodilatation locally, or by release of epinephrine from the adrenals when given intravenously. Significantly, stimulating the sympathetic nerves may reduce sweating, possibly by producing vasoconstriction and thereby reducing the local epinephrine level. Conversely sympathectomy is followed within an hour by free sweating, presumably due to the effect of vasodilation permitting more adrenalin to reach the gland. It is now clear that sweating in the horse is humorally controlled. In exercise the blood epinephrine increases by as much as 78 per cent. This initiates sweating. "Dry coat," or anhidrosis, results from prolonged stimulation of the sweat glands by epinephrine, secreted as a response to conditions of hot moist climate. The sweat glands become accommodated to the raised epinephrine content of the blood, so becoming insensitive to it. This creates a vicious cycle, only to be broken by rest in a cooler climate.

Shelley & Hurley have explored the physiology of the apocrine sweat gland in man (95 to 107). In man, the apocrine sweat glands are found in greatest abundance in the axillae. Here they are mingled with the eccrine glands so that the apocrine secretions may readily be lost in eccrine sweat. Accordingly, two criteria are desirable for any study of apocrine function. First, eccrine response must be minimal and not spontaneous; second, the individual selected must have well developed apocrine glands. Negroes

have generally proven to be the best subjects. In any subject with diminutive apocrine glands, research on apocrine responses is ill-fated. It was the authors' similar experience in the field of sebaceous gland physiology. Studying the small version of the gland is taking on a crippling handicap. In man, apocrine sweat is a white, gray, or at times faintly yellow fluid which generally appears at the orifice of the hair follicle. This lactescent droplet dries to form a glistening glue-like mass, if not diluted by eccrine sweat. Apocrine sweating can be produced by any severe emotional stress such as fear or pain. Conversely, heating does not induce apocrine sweating unless the subject is emotionally upset by the environmental stress. The gland is responsive to epinephrine, norepinephrine, or oxytocin, either locally or intravenously. Following such a response there may be a refractory period. Acetylcholine or pilocarpine locally were without effect except in a few subjects. An apocrine sweat response could not be demonstrated in children before puberty, and in elderly subjects it was decreased. The amount of apocrine sweat which could be produced was but a tiny fraction of the eccrine response.

Study of the myoepithelial sheath *in vivo* revealed peristaltic waves taking place in response to stimulation. It was, moreover, possible to demonstrate that the apocrine gland was a reservoir of sweat which could be milked manually. Once the contents were expressed one to two days elapsed before the gland could be made to respond again. It was postulated that eccrine secretion is a continuous process whereas apocrine sweating is the delivery of preformed sweat to the skin surface as a result of peristaltic contractile waves produced by the myoepithelium responding to mechanical stimulation (frictional motion), stretch reflex (overfilling of gland), neural stimuli (adrenergic fibres), or humoral (circulating epinephrine) stimuli.

It was possible to show nerves supplying the gland and furthermore that these nerves were not cholinergic. Thus, the striking contrast of eccrine and apocrine glands was complete. Further studies were done showing that apocrine sweat is odorless and sterile as it reaches the skin surface. Within hours, however, the surface flora attack the apocrine sweat producing the characteristic apocrine odor of the axilla. The problem of colored apocrine sweat has also been a subject of study.

Finally, the apocrine glands of the ear canal were studied and found to show the same physiological responses as those of the axilla.

ECCRINE SWEAT GLAND

The eccrine gland has been extensively reviewed in the past six years. Kuno's long awaited monograph (108) on human perspiration appeared in 1956. It contains a comprehensive account of the physiology of sweating and provides much of the Japanese literature otherwise lost because of the language barrier. Randall has presented a pair of review articles (109, 110) which exquisitely detail all of our knowledge concerning the physiology and pharmacology of the sweat gland. In addition the Robinsons have presented reviews both on sweat gland research methodology and on the chemical

composition of sweat (111, 112). These five reviews coupled with those in Rothman and Montagna's works (1, 2) provide anyone entering the field a truly superb background.

In the field of the chemical composition of the sweat the literature is so extensive and controversial that one must refer to the Robinsons' review (112). From all recent work one single observation stands out as significant and unchallenged. This is the finding of Di Sant'Agnese *et al.* (113) that the electrolyte composition of sweat from patients with cystic fibrosis of the pancreas is abnormal. This group, in a study of 43 patients, found the sodium and chloride concentration was two to four times that found in controls; for example, the chloride ranged from 60 to 160 m.eq./liter, whereas the control values all fell below 80 m.eq./liter. It is significant that these patients tolerate hot weather poorly, some showing vascular collapse, hyperpyrexia, and coma. It is Di Sant'Agnese's feeling that the excessive saline loss contracts the extracellular space, thus leading to heat intolerance. The sweat electrolyte pattern determination is now used as a diagnostic procedure to distinguish cystic fibrosis of the pancreas from other clinically similar problems. As to the cause of the sweat gland change, observers (114) feel that this gland is simply one of the many glandular organs which are involved in this system process which they would term mucoviscidosis.

An increasing body of evidence is being produced to support the view that the eccrine sweat duct is far from a passive excretory duct (108, 115 to 119). Some of it is speculative, to explain chemical changes which occur during secretion of sweat. Some of it results from observing histochemically that the luminal cells are metabolically active. These cells are basophilic, heavily laden with disulfide groups, abound in succinic dehydrogenase and, finally, they contain glycogen which disappears during active sweating. Unfortunately the direct cannulation procedures Richards used in the renal tubule are not feasible for use in the sweat gland.

Randall, Hertzman *et al.* (120, 121) have continued their fundamental studies on sweating. They have discovered that sweating in response to a gradually rising environmental temperature shows dermatomal recruitment. The first area to respond was the dorsal surface of the foot, next the calf, the thigh, thence the trunk, upper extremity, and finally the face. Sweating on the foot and calf may be profuse while the chest, face, and upper extremity remain dry. In yet another study they have found that there are marked variations in the sweating patterns of different patients or in the same patient when stimulation is applied at different levels of the sympathetic trunk. Working with man, they actually simulated the lumbar sympathetic trunk with a current of the following characteristics: frequency, 20/second; duration 5 msec.; voltage, 1 to 50. In no instance was there any crossover response. Furthermore, they found local disparity of the sudomotor, pilomotor, and vasomotor response. It was their conclusion that the sympathetic supply is so anatomically randomized that one must take great care in interpreting all of the literature on the effects of sympathectomy.

The fact that epinephrine will cause an eccrine response in man has con-

tinued to intrigue students of sweat gland physiology (122 to 126). It seems apparent as a result of recent work that the neural supply of all human eccrine glands is definitely cholinergic. Even the palmar glands have been shown to have cholinergic fibres about them, and to be blocked by atropine despite the fact that they show responses to emotional stimuli. Nevertheless, epinephrine does act on the same glands as respond to heat or acetylcholine. It would seem that in unusual circumstances a high blood level of epinephrine might act humorally on the gland. However, the sweating of hypoglycemia and in association with pheochromocytoma is not due to the circulating epinephrine acting on the gland, since the response can be abolished locally with atropine. Thus, in contrast to the findings for the horse, there is no evidence to indicate that epinephrine plays a significant physiologic role in sweating in man.

Sweating rates decline in the presence of a prolonged thermal stimulus. Several investigators have found that by the fifth hour of exposure to an environment of 40°C. with 50 per cent relative humidity, the sweat rate may decline as much as 60 per cent. Studies have revealed that this decline is primarily due to a reduction in the output of each gland and to a lesser extent a reduction in the number of active glands (127). It is not caused by a central nervous system change, since apparently the gland itself fatigues. In any event, it has been possible to show that the response of the gland to chemical stimulation is depressed well below normal. Furthermore, recent histologic and histochemical studies (128) show that after profuse sweating the secretory cells show loss of glycogen, decrease in granules, vacuolization, atrophy, and pyknosis. The nuclei become distorted, present an increase in chromatin and show a thickened membrane. These findings provide histologic proof of a morphologic basis for sweat gland fatigue.

Finally, Lee (129) has provided us with an informative study of the physiology of gustatory sweating. In temperate zones gustatory sweating is unusual because the reflex stimulus of spicy foods is usually insufficient to initiate sweating in the resting gland. In the tropical environment in which Lee worked, the sweat glands are highly responsive and therefore reveal the gustatory reflex clearly. Using chilies (*Capssicum minimum*) as a stimulus, 45 of the 46 young male subjects showed a symmetrical sweating response confined to the head and neck. The exact loci varied, but invariably the sweating was associated with flushing of the face and upper body, salivation, lacrimation, and nasal secretion. The reflex sweating showed recruitment, facilitation, and fatigue. Lee showed that the receptor arc of the reflex was composed of pain fibres, whereas the motor arc was cholinergic sympathetic fibres.

NERVES

The general problems of cutaneous sensation have received the close attention of two careful critical reviewers (130, 131). In addition, reviews on the specific problems of warmth sensation (132), and pruritus (133, 134)

have also been published. Finally, a monographic review of the neuroanatomy of the skin has appeared (135). A survey of the reviews and the published research papers clearly indicates that cutaneous sensation has accounted for essentially all of the neurophysiology of the skin.

It has been a period of revolution and the Oxford group led by Weddell have revolutionized modern thinking in regard to the doctrine of specific energy. They have produced a wealth of experiments and papers (136 to 147) to prove that cutaneous sensation in man is not subserved by specific nerve endings as has been taught for several generations. One must read the review of Sinclair to appreciate the flavor and force of their arguments, but here we will summarize the salient features. The classic belief has been that there are four specialized encapsulated endings, each representing a sensory modality. The Oxford group have shown that temperature sensibility is adequately represented in the pinna of the ear, despite the complete absence of organized endings of any kind. On the lip the temperature thresholds of the mucosa are similar to those of the skin margins, although the mucosa has many organized endings and the skin has none. Again in the cornea, possessing only free nerve-endings, subjects are able to perceive touch, warmth, cold, and pain. They conclude that the unencapsulated nerve-ending in the skin can give rise to a wide range of sensory experience when suitably stimulated.

Next they have rejected the theory that specific nerves exist; in rabbits stimulation of a single hair actuated fibres of different sizes and thus different conduction rates. They feel activity in a single fibre is an unnatural event which if it ever occurs probably does not produce sensation. Reaction times which have been used to distinguish specific fibre groups are discarded by the Oxford workers as failing to give any idea of conduction time. They argue that we may not assume touch impulses travel faster than pain simply because we perceive touch first after stubbing our toe. They point up the possibility of many other factors being determinants of reaction time. For them second pain is an artifact.

They have critically and experimentally surveyed the effect of reversible blocks on peripheral nerves. Here they reluctantly conclude that fibre size is a major factor in the production of sensory dissociation. But again in the sensory dissociation of sectioning of nerves, roots or cord, as well as regeneration studies they find little evidence for specific fibres. They conclude that the specific fibre theory of Von Frey is not a realistic view of current knowledge. All their data and reasoning flow in favor of a pattern theory in which cutaneous stimulation produces a complex spatially and temporally dispersed pattern of impulses which passes up the cord to the sensory cortex. This pattern can be continuously variable and therefore account for the range of sensations which can be aroused in the skin. Oxford has thrown down the gauntlet. We may expect someone somewhere will pick it up.

Certainly others (148) have confirmed the Oxford group's finding that encapsulated nerve endings are not a *sine qua non* for the full range of cu-

taneous sensation. Also from the psychologists (149) comes evidence that double pain is an artefact. Positive reports of double pain are reviewed as being due to inadequate experimental control of the stimulus, psychophysical methods, and to lack of statistical analysis.

Still others relate specific stimuli to the firing of specific size nerve fibres in the skin of the cat and toad (150). In the cat, the touch fibres proved to be from 8 to 14 μ . in diameter, the cold fibres 1.5 to 3 μ ., whereas the nociceptive ranged from 3 to 11 μ . Landau & Bishop (151) have marshalled further evidence in man that the fine unmyelinated C fibre endings are responsible for the delayed persisting and burning type of pain characteristic of partial nerve lesions and of tabes. Inflammatory subcutaneous pain is assignable almost entirely to activation of C fibre endings, which they feel are specifically sensitized by inflammation.

Pruritus, long neglected by the sensory physiologists, has recently been the subject of intensive study by several groups. Cormia & Kuykendall (152 to 154) used histamine as a stimulus for pruritus. Normally, the threshold dilution of histamine for the production of itching was about 10^{-5} . Area differences in threshold as well as individual differences were seen. They found that the itch threshold was greatly lowered at night and in areas of dermatitic skin. Furthermore, psychic trauma consistently led to a lowering of the itch threshold. It was their conclusion that burning is a severe form of itching. When an initial intradermal injection of histamine produced pruritus, two additional injections at the same site commonly resulted in burning. Further injections could lead to a refractory site which remained insensitive for hours.

Shelley & Arthur (134, 155 to 157) have discovered evidence for an enzymatic basis for itching. Stimulated by the observation of Broadbent (158) that cowhage itch powder (*Mucuna pruriens*) could be inactivated by boiling, they isolated the active pruritogenic component of cowhage. Further study by them revealed it to be a proteinase. It was then found that all endopeptidases, either of plant or protein nature, which were active at a pH of about 7, produced pruritus when introduced into the skin. Surprisingly, small quantities of very dilute crystalline proteinase would produce pruritus in the absence of any clinical changes. For example, 0.01 ml. of 10^{-5} trypsin produced pruritus of several minutes duration. Great differences in threshold were found for different areas and different subjects. Dermatitic skin proved to be sensitive to solutions as low as 10^{-7} . As a result of this work, it has been postulated that the endopeptidases are the chemomediators for pruritus and that these are released or activated in tissue as a result of trauma. The sources include the epidermis (cathepsins), capillaries (plasmin), infiltrates (leucoprotease), as well as bacterial and fungal proteases.

Further studies then revealed that the skin has punctate areas of high sensitivity and that these "itch points" show a rich aggregate of fine sub-epidermal nerve fibres. Physiologically, the itch point is simply a low

threshold area for chemical, mechanical, thermal, and electrical stimuli for itching. Evidence was brought forward to show that the stimulation of any itch point is followed by a minimal delay period corresponding to the time required for a nerve impulse to travel up the peripheral nerve at the rate of 2 m. per sec. This is consonant with the view that itching is subserved by fine unmyelinated C fibres only.

Cutaneous pain in man has been the object of study by another group headed by Armstrong (159, 160). They have used the base of a cantharidin bulla as the test object, applying numerous chemical compounds in a search for the identity of the pain-producing substance in plasma and inflammatory exudates. Among their findings are the following observations:

Acetylcholine, KCl, hypotonic, hypertonic, and acidic solutions all produce pain. Histamine and histamine liberators produce pruritus as well as pain. Other pain producing agents include blister fluid serum, platelet extract, serotonin, creatinine, and tryptamine. They have strong evidence that the pain producing substance in blister fluid, as well as other exudates, is a polypeptide possibly resulting from proteolytic enzyme action occurring during tissue damage. The close relationship of this work to that of Shelley and Arthur's on pruritus is apparent.

The problem of sensitization of the nerve endings to stimuli is a significant one and two workers have experimental data on this point. Skouby (161) has extended his previous investigations, now showing that pain receptors in the human forearm can become more responsive in the presence of methacholine and acetylcholine. Lowenstein (162) in studying isolated frog skin found that in stimulation of the sympathetic nerves there was a distinct lowering of the threshold for the firing of touch receptors. He found that he could reproduce the effect with epinephrine or norepinephrine, but not with acetylcholine. One must wonder if this finding has any bearing on the tingling sensation experienced during a sudden emotional stress.

Cauna (163) has described the mechanics of the ridge on the palms and soles as a highly efficient mechanism for the selective transmission of touch stimuli to deeply placed nerve receptors. Sidman & Fawcett (164) describe the great increase in lipides which occurs in the brown adipose tissue of a mouse after denervation. Furthermore, this denervated interscapular fat fails to show any depletion of its lipide during an extreme fast, in contrast to normally innervated brown fat. The trophic implications of this neurologic effect invite further study.

Finally, attention is drawn to the recent work on the pilomotor axon reflex (165 to 167). In an extensive study, Brenning has shown that this axon reflex can be elicited by faradic stimulation or by the introduction into the skin of acetylcholine, nicotine, or lobeline. He feels that neither the sympathetic nor the sensory nerve fibres are involved in this reflex, but rather he postulates the presence of a distinct pilomotor axon reflex fibre with a separate trophic center outside the sympathetic chain.

BLOOD VESSELS

Recent advances in the field of cutaneous circulation have been marked by a revival of interest in the venous system. It has been known for some time that a temperature gradient exists between the arteries and veins of the extremities (168), and it has been speculated that, in addition to other established factors, the existence of complex longitudinal and circumferential temperature gradients on the surface of the forearm may be accounted for by a varying rate of flow in the subcutaneous veins (169).

These speculations imply selective contractile activity of the veins. It has long been known that cutaneous venules do undergo rhythmic changes in diameter, that they possess smooth muscle and a nerve supply, and will contract in response to epinephrine (170). That this venous contractility may be independent and selective has been established by recent investigations. Haddy *et al.* (171), working with dogs, compared the blood pressure in the small and large veins of the leg. They were able to show that the pressure in the small veins could vary by as much as 40 mm. Hg, independent of any change in blood pressure in the large veins.

Duggan *et al.* (172), working with human subjects, studied changes in the pressure in superficial veins temporarily isolated by nonsurgical means. Using procaine injected proximally and ganglionic blocking agents, they were able to show that the pressure in the veins was mediated by the nervous system.

Following this work, Kelly & Visscher (173) reported on their experiments with the small cutaneous arteries and veins of the extremity in the dog. Their cannulation system and controls were designed to rule out cardiac output, back-pressure from the central venous system, or skeletal muscle contraction as factors controlling intravascular pressure changes when the sympathetic nerves were stimulated electrically. They recorded independently variable pressure increases in small arteries from the control average of 99 mm. Hg to 138 mm. Hg upon stimulation, and in the veins from 17 mm. Hg to 30 mm. Hg. When stimulation was stopped, pressure in the small arteries fell rapidly to an average of 55 mm. Hg before returning to normal. While, in the small veins, after stimulation ceased, the pressure rose to an average of 42 mm. Hg before returning to normal. They were also able to show that a marked increase in the pressure in small veins lasted as long as 10 min. after the end of respiration and cardiac arrest. This recently presented experimental evidence that the small veins have a contractility independent both of the large veins and the arterial system but under control of the nervous system gives a new importance to the venous circulatory system in studies of blood pressure and thermal exchange.

The problem of heat exchange at the skin surface has been of great interest to many workers in the field of vascular studies. Many of the technical problems involved are reviewed by Hertzman (174), who emphasized the complexity of the factors entering into such studies. From recent investiga-

tive work, that of Glaser & Newling (175, 176), stresses the importance of the influence of thermal preconditioning of human subjects in relation to the skin temperature at which thermal equilibrium is subsequently established. In one series their subjects were exposed at controlled barometric pressure to a temperature of 27° to 30°C. until thermal equilibrium was established. They were then subjected to 5 min. of either warming or cooling, following which they were again allowed to reach thermal equilibrium, either at a temperature of 16° to 18°C. or 38° to 40°C. It was found, in either case, that the skin temperature at which final equilibrium was reached varied in accordance with the 5 min. precondition, independently of the final (equilibration) temperature to which they were exposed a much longer time. If they had been pre-warmed for 5 min., the equilibrium skin temperature was relatively higher than if they had been precooled for 5 min.

The role of temperature pre-conditioning in direct relation to neurovascular responses has recently been illuminated by Davies' *et al.* (177) work with the Raynaud phenomenon. This condition is characterized by spasm of the digital arteries in response to cold exposure with attendant color, temperature, and sensory changes. The subdivision of the disease studied by these workers (pneumatic hammer disease) occurs in workers who use pneumatic tools delivering blows at a rate in excess of 1000 per minute. They found that, while in England, 81 per cent of riveters and 61 per cent of caulkers using such tools showed evidence of the Raynaud phenomenon; in Singapore where the average temperature varies only between 71 and 91°F., the disease does not exist among such workers. They confirmed this clinical observation by challenging 30 such workers with controlled cold exposure of the hands after a prior 75 min. general thermal equilibration at 51°F. None of the workers showed the Raynaud phenomenon, but two of three known Raynaud reactors (controls) did. The third control subject is particularly interesting in that, although he had exhibited the phenomenon a few days previously in England, he failed to exhibit it under these test conditions. This subject had, by chance, been preconditioned before challenging by only 15 min. general equilibration at 51°F.

Sir Thomas Lewis' generalized theory that all cutaneous inflammatory processes result from histamine liberation has been previously objected to by comparing the triple response to the ultraviolet light reaction (178). In the latter, whealing is absent, pallor is absent, the duration and latent period are longer, and flare, which rarely occurs, extends only very slightly beyond the border of the exposed area. These arguments against Lewis' theory have been reinforced by the work of Partington (179). In human subjects, he found that histamine introduced by pricking caused the same response in areas of ultraviolet erythema and normal skin. The use of histamine liberators to reduce the skin histamine content before and after ultraviolet exposure did not affect the erythema. Pretreatment with antihistamines reduced the skin response to histamine but not to ultraviolet. In the rabbit, histamine did

not produce erythema, but ultraviolet light did. This new evidence seems conclusive that the vasodilator principle in ultraviolet light erythema is not histamine, and that Lewis' general theory is not applicable to all cutaneous inflammatory states.

The remarkable therapeutic efficiency of ACTH, cortisone, and its variants in inflammatory cutaneous disease is now a matter-of-course clinical observation. This property of these drugs is distinguished as an entity apart from their influence on the antigen-antibody reaction. They have no direct effect on histamine itself. That these steroids do have a direct action on the vascular system was demonstrated by Levine and others (180, 181). Using the Chambers-Zweifach preparation of the rat mesentery, they were able to observe directly the action of drugs on the small vessels exposed under the microscope. The subjects were adrenalectomized rats treated with DOCA. When norepinephrine was added to this vascular preparation, the contractile response of the vessels was normal at first, but soon decreased so that after 1 to 2 hr., 10 to 15 times the initial dosage of norepinephrine was required to produce the same response. They found, however, that if cortisone was then added to the preparation, it would soon respond to the initial dosage of norepinephrine. They postulated that the contractile response of the vascular system to norepinephrine depends on the presence of cortisone. Ebert & Barclay (182), using a chamber to observe directly a revascularized area of the rabbit ear, were able to demonstrate that the vascular tone was increased by cortisone in normal skin. They were also able to demonstrate that cortisone possesses a tonic vascular effect not only in inflammatory states caused by antigen-antibody reactions (tuberculin) but also in inflammation of nonspecific (low-grade bacterial infection) origin.

CONNECTIVE TISSUE

The studies of Follis (183, 184) on the rare pediatric disease, osteogenesis imperfecta congenita, have emphasized that it is not only a disease characterized by blue sclerae and failure of bone maturation, but by generalized connective tissue disturbance. He studied the skin in five cases. He found a normal epidermis, but an unusually thin corium. The corium had the filamentous, reticular appearance (H and E), and the selective staining qualities (took Gomori silver impregnation, Schiff-positive) of reticulum, comparable in appearance to that of a three-month embryo. No evidence of collagen formation was noted. Since reticulum tissue is now identified as immature collagen (185), the further study of this disease of failure of maturation of dermal connective tissue should be profitable to our basic knowledge of collagen formation.

When the effectiveness of steroid therapy in the "collagen diseases" was recognized, many investigators began to search for the basic mechanisms involved. The inhibitory action of these drugs on fibroblastic activity was soon established, and the mechanism of their influence on the vascular

system is dealt with elsewhere in this chapter. That part of this therapeutic effect might be due to steroid influence on ground substance formation (186, 187) has been reviewed skeptically by Dorfman (188, 189). He points out that the doses of cortisone employed to demonstrate decreased utilization of S^{35} and $S^{35}O_4$ in rat skin are very high (20 mgms. per Kg.), and cites, as an example of the pitfalls in this type of research, work from his own laboratory in which another drug, testosterone, was shown to cause a marked increase of hexosamine content in the chick comb, but not in chick skin. He believes that the influence of cortisone on the vascular system may, indirectly, play a dominant role in ground substance formation.

The influence of cortisone on the action of hyaluronidase has been the subject of much research. Since Opsahl (190) reported on an inhibitory action of cortisone on hyaluronidase, a large and contradictory literature has appeared which Barer (191) has tabulated as increasing, decreasing, having no effect, depending on dosage, and depending on the subject. In her own admirably conducted experiments, she reported that cortisone did not influence the action of hyaluronidase in rabbit skin.

The studies of Barer (191 to 193) and others have supplied valuable basic information on what happens when a fluid is injected intradermally. An ingenious technical arrangement made it possible to record photographically and simultaneously the pressure of injection (velodyne motor, manometer, oscilloscope) and the spread of the injected fluid (thorotrast, cineradiography) in the rabbit. Shortly after the beginning of a constant-rate injection, the pressure was found to rise to a plateau. The plateau level was found to increase with the rate of injection. For example, a plateau pressure at a constant injection rate of 0.06 ml. per minute was 149 mm. Hg, while at a rate of 0.3 ml. per minute, it was 289 mm. Hg. With a syringe of 53 mm. bore, operated rapidly by hand, the mean pressure of injection was found to be 300 mmg. Hg. While the actual pressure was shown to vary with rate rather than volume, a volume of injectant as low as 0.0004 ml. was found to be required if intradermal pressure was not to be significantly elevated. In one series of experiments, the thorotrast fluid was injected with and without hyaluronidase. With constant rate injection at a given speed, hyaluronidase was shown to spread the injectant (in 15 to 150 seconds) over an area about twice the size of the control. The control sites reached a maximal spread at 90 min., while the hyaluronidase-treated sites continued to enlarge for several hours. These latter findings refute the arguments of Hechter (194) who felt that hyaluronidase action was dependent on pressure and volume factors.

Juhlin (195 to 197) has contributed several new facts to the literature of hyaluronidase. His work was conducted with newly killed rabbits to eliminate the influence of vascular factors. Injecting bacterial hyaluronidase again six hours after the initial injection, it was found that a hyaluronidase-resistant "tissue barrier" had formed. Testicular hyaluronidase injections produced the same "barrier," but to a lesser degree. The tissue response to

both hyaluronidases was found to be normal after 24 hr. His proposal that heparin may have formed a hyaluronidase-resistant complex with mucopolysaccharides and proteins needs further confirmation.

In other experiments, hydrophilic and lyophilic spherical particles of mean diameter $0.1\ \mu$. and $0.75\ \mu$. (viral and bacterial models) were injected with and without hyaluronidase. The enzyme was found to greatly increase the spread of both types of particles of $0.1\ \mu$., and of the hydrophilic particles of $0.75\ \mu$. size. However, the lipophilic $0.75\ \mu$. particles were found to spread in a larger area in control than enzyme-treated sites. The functional size of pores limiting lateral spread in hyaluronidase-treated skin was found to be between 0.1 and $0.6\ \mu$.

The mast cell, first described by Ehrlich 75 years ago, has been the subject of much attention since it was identified as the heparin producing agent of the body (198). This granular, metachromatically staining cell is characteristically concentrated in the loose reticular tissue surrounding small blood vessels and underlying epithelial, serous and synovial membranes. Yet, in the rabbit, the majority of these cells are found in the blood, while they occur in numbers in the parenchyma of the liver in the dog. This latter species specific location furnished Riley (199 to 201) with an important clue leading to the identification of the mast cell as the histamine producing and releasing agent. When peptone is injected intravenously into the dog, a true histamine shock ensues with incoagulability of the blood due to simultaneous heparin release. Both histamine and heparin in this species were shown (202) to be released principally from the liver. However, when histamine is injected intravenously in the dog, histamine shock occurs without incoagulability, i.e., heparin is not released. Nor does the injection of heparin cause the release of histamine. These circumstances suggested, then, that peptone acted to release both histamine and heparin at a common site, the liver, where in the dog the mast cell was known to be concentrated.

A second clue was furnished by the dermatological disease urticaria pigmentosa. In this condition, focal collections of mast cells in the skin just beneath the epidermis respond to mechanical stimulation with the "triple response" (urticaria) of Lewis, the classic cutaneous sign of histamine release.

In addition to Riley (203, 204, 205), a number of other investigators have rapidly accumulated a mass of evidence correlating the mast cell with histamine production and release in the skin and other tissues. The mast cells of the rat have been shown to be disrupted by histamine liberators. The histamine content of various tissues has been shown to correlate with the concentration of mast cells in those tissues. In pigs and cattle, the skin has been shown to have more histamine in the upper than the lower part of the ear and more at the tip than the base. This corresponds directly to the mast cell distribution. The skins of man, mouse, rat, cat, dog, guinea pig, and rabbit have been divided into dermal and epidermal layers and the pattern of histamine and mast cell distribution found to correspond to this division.

With cat skin, frozen and sliced parallel to the surface into successive layers, a histamine-mast cell correspondence has been found to exist in each successive layer. A lesion of urticaria pigmentosa has been found to contain as much as 950 $\mu\text{g./g.}$ of histamine. Dog mast cell tumors have been shown to have as much as 594 $\mu\text{g./g.}$ of histamine. An average value of 7 $\mu\text{g.}$ of histamine per mast cell has been proposed. The histamine content of mast cells has been shown to vary directly with their size. The intracellular concentration of histamine is about 0.1 M.

Evidence is collecting which identifies the basophilic leukocyte as a circulating intravascular mast cell (206, 207, 208). Much of the blood histamine has been shown to be present in these basophils. Heparin has been shown to be a constituent of the basophilic cells in basophilic leukemia.

The function of the mast cells seemed further enlarged when recent work (209, 210) identified serotonin in the mast cells of the rat (peritoneal washings and skin). However, Sjoerdsma *et al.* (211), working with mast cell tumors of mice, dogs, and urticaria pigmentosa in man, were able to confirm the presence of serotonin only in the mouse. Species difference apparently accounts for its presence in rats and mice, and its absence in dogs and man.

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TEMPERATURE COMPENSATION^{1,2}

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The independence from the effects of temperature on the rate of chemical reaction which organisms have achieved has become more and more of interest to physiologists as well as ecologists. Prosser (1) has recently pointed out the basic principles whereby the organism is able to achieve a harmonious relation with the physical and chemical stringencies of its environment and this review will be organised according to the classification that he has proposed. Prosser considers the more primitive response is an adjustment of the metabolism of the organism to facilitate its activities in a variety of environments. At a higher level of evolution the internal milieu is regulated, and life is then freed from the restrictions of the environment at the price of paying the capital and current cost of organic regulation.

There is no abrupt passage from adjustment to regulation in the evolutionary scale. For example it is likely that all motile organisms have some behavioural regulation with respect to temperature (2). In a situation where the response to temperature is not overruled by the compulsion to respond to some other environmental identity, say humidity (3), animals will move away from situations where the stress of temperature impinges on them to too severe a degree. The range of thermal indifference may be large for a well-insulated animal (4) but it would not appear likely to be infinite. Again, even this most primitive regulatory response has coupled with it a large measure of adjustment. The thermal preferendum will often change markedly with the thermal history of the organism. The carp, for instance (5), which if left continuously in a temperature gradient would be found almost exclusively within a few degrees of 32°C., will select temperatures between 15° and 17° when placed in a temperature gradient after a sufficient sojourn at 10°C.

Certain cases of more or less constancy of activity of poikilotherms in the face of changes in temperature should probably not be considered as instances of temperature compensation, or rather, perhaps it should be said that to achieve a given constancy probably requires less compensation on the part of the animal than might be expected without consideration of the circumstances. The simplest of these cases is that of rate of movement. In animals, as in mechanical situations, movement is resisted by viscosity and the rate of energy production required varies as a power of the speed of the movement (6). In the fish the requirement for metabolism available for

¹ The survey of literature pertaining to this review was completed in July, 1957.

² I am indebted to the National Research Council of Canada for funds to support an assistant in the preparation of this review and to Miss Phyllis Lum for her conscientious work in this role.

activity goes up in proportion to the square of the swimming speed that can be attained [ref. (7), Fig. 25]. Thus, although the rate of the metabolism which can be provided for the activity of movement may have a Q_{10} of 3.4 for example, as it does in the goldfish from 10° to 20°C. (8), the Q_{10} for the change in the swimming speed over the same temperature range is 1.7 (9). Again, temperature is not by any means always the environmental identity that governs the rate of the organism's activity. For aquatic organisms, for instance, the concentration of oxygen may frequently limit the metabolic rate when they are active at high temperatures so that the level of performance may not change or may even decrease for this reason alone (e.g., 10). Such questions as these will not be dealt with here but a first explanation of any homeostasis can probably be best sought among them and certainly the relation of organisation to active metabolism needs to be much more thoroughly explored than it has up to the present time.

The temperature-independent rhythms of poikilotherms (11, 12, 13) will also be excluded from further consideration by mentioning them here. These must operate without the need for the adjustment of the metabolic rate and indeed in spite of any such adjustment, except for the shift in the biokinetic range which acclimation brings about. As yet there is no theory which satisfactorily accounts for these extreme constancies which must be achieved surely by some coupling of the excited state and the basal state of whatever organ it is that governs the response.

There are essentially three levels on which the organism achieves adjustment or regulation as the environment changes. At the most immediate level there may be day to day changes in its systemic and cellular organisation in direct response to changes in a given identity, in the present case temperature. Next there may be long-term responses operating throughout the life of the individual and indeed possibly influencing a succeeding generation through nongenetic parental influence. These effects of the thermal history may be coupled with the results of the conflicting or reinforcing influences of other environmental identities such as the photoperiod, salinity or humidity. Finally the process of compensation must be considered on the phylogenetic level at which the mechanisms both for the production of direct responses to temperature and the mechanisms for the anticipation of seasonal changes have been fixed in the heritage of the species.

These three levels of response will be recognised in the present review by attaching to them the somewhat arbitrarily imposed terms respectively of "acclimation," "acclimatization," and "adaptation." To use these terms with these particular and restricted meanings is to run counter to the choice and judgment of many able workers in the field but something must be done for the sake of clarity in thinking and in communication between two somewhat alien groups, the physiologists and the ecologists. It is unfortunate that the English language has not developed a consistent terminology for these processes. The greatest conflict in usage has been with regard to the term "adaptation." To the biologist this term has been associated with

phylogenetic change for almost a century. The physiologist, on the other hand, appears to have almost exclusively applied the word to changes of the phenotype within its reaction norm, as for example Selye's "general adaptation syndrome." Otherwise the word has been used indifferently to designate all three levels of response distinguished here, occasionally all within the same article. The terms "acclimation" and "acclimatization" have not in general been applied by anyone to the phylogenetic level and have been used with the restricted meanings it is proposed to follow here, or interchangeably.

A number of excellent reviews touching on various aspects of the effects of temperature on organisms to which reference will be made in the appropriate sections, together with the book of Precht, Christophersen & Hensel (14) have greatly simplified the problem of writing this article and reduced the need for an extensive bibliography. While key references will be repeated here, most of those given adequate notice by these authorities will be omitted.

ADJUSTMENTS

There are two avenues of successful thermal adjustment open to the poikilotherms. These are decided by the two major divergent habitats, land and water. On land, with the existence of great differences in the annual minimum fluctuations in temperature in various regions, adjustment for the animal which does not regulate its body temperature often requires an ability to withstand freezing temperatures. At temperatures above freezing the prime variant from the poles to the equator is the length of the season for activity, not the highest temperatures reached. In the most severe of the hot environments most animals find refuge in some microhabitat from the greatest heat, which comes from solar radiation and is effectively blocked by shade. The terrestrial poikilotherm has diverged mainly according to its geographic distribution by evolving means of withstanding various degrees of subfreezing temperatures. In the sea there is a much more definite gradient in the highest temperature reached in the annual cycle and the lowest temperature is ordinarily above 0°C., at least in estuaries. It is to the advantage of the aquatic organism then, to be able to adjust its activity in a compensatory fashion to meet the prevailing temperature of its habitat. Christophersen & Precht (15) have characterised these two extremes of adjustment by applying to them the term "resistance" compensation and "performance" compensation.

Acclimation.—The lability of the thermal adjustment of organisms is well recognised and most investigators now stabilise the thermal histories of their subjects before experimentation. The lethal temperatures of fish, for instance, as determined by various criteria, show such variation with thermal acclimation as to be of little value without an accompanying definition of the state of the thermal adjustment of the sample on which the determination was made (16 to 21). Similar, but in some cases less marked

adjustments as shown by the lethal temperature have been reported for amphibia (22), insects (23 to 28), annelids (29), and bacteria (30 to 32). The adjustment of the lethal temperature of the lobster (33) is as marked as in fish and the same is probably true for the crayfish (34). These adjustments, when determinations of both upper and lower lethal temperatures have been made, have been found to be in the direction which placed the biokinetic range more symmetrically about the acclimation temperature.

Thermal adjustment has also been widely examined by measuring the activity or the metabolism of the animal or its organs. A review on this specific subject was recently published by Bullock (35) and the matter has received extensive attention in Precht *et al.* (14). Only supplementary references are therefore added here. The results recently found are typical of the earlier work. Suhrmann (36) measured the metabolism of the crucian carp immobilised by spinal section. The rate of oxygen consumption of warm-acclimated fish (26°C.) was about 30 per cent lower than that of cold-acclimated ones (5°C.) at all temperatures from 10° to 30°C. Results of a similar nature and magnitude have been recently reported for the oxygen consumption of the American cockroach (37), aquatic insect larvae (38), and a crab (39), and for the rate of heartbeat of a limpet (40). The extreme of thermal adjustment appears to have been found in lizards (41, 42). Two Dalmatian species were found to adjust to such an extent that their standard rate of metabolism was the same at 15° as at 25°C. when they were fully acclimated to each respective temperature.

When temperatures are too extreme, irregularities in the response of metabolism are found (27, 43).

Measures of activity of the whole organism have also been found to display a compensatory response to temperature changes. In the goldfish (9), except for the highest temperatures, thermal acclimation allows the fish to swim as well or better than they would otherwise be able to do at that temperature. Thus the adjustment permits a gain of activity.

Acclimatization.—The direct effects of temperature that bring about thermal acclimation may be opposed or reinforced by the effects of other environmental identities. Thus, the thermal resistance of the gill tissue of the mussel depends on the calcium content of the water in which it has been living as well as upon its thermal history (44). With regard to seasonal effects Sullivan & Fisher (45) found that the thermal preferendum of the speckled trout increased suddenly in early spring although there was no substantial change in water temperature at that time. Hart (46) reported a number of inconsistencies in the lethal temperatures of fish acclimated to the same temperature at different seasons. Rainbow trout (47) and planaria (48) have a higher thermal resistance in summer than when acclimated to the same temperature in winter. Hoar (49) found an upward shift in both the lower and the upper lethal temperatures of goldfish in summer as compared with winter. He was later (50) able to produce effects of the same magnitude by subjecting his fish respectively to 16 and 8 hr. periods of light daily. He also noted that summer fish could be made more

resistant to low temperatures by treating with thiourea, whereas winter fish at the same level of thermal acclimation gave no clear response when so treated.

The magnitude of the concomitant effects of the other environmental identities on thermal adjustment so far found have not ordinarily been large except for Kirkberger's data (29) for the annelid, *Eisenia foetida*, for which she reports a spring-autumn shift of the lethal equivalent to a drop of some 10 C.° in acclimation temperature. A comparison of Hoar's data for the seasonal and light effects with other data for the thermal resistance of the goldfish [ref. (51), appendix] shows the extra shift to be equivalent to that brought about by a difference of 4 C.° in acclimation temperature. While the evidence is not conclusive, it would appear that the adjustments of the rate of heartbeat of the limpet, *Acmaea* (40), can also be almost entirely accounted for by changes in acclimation temperature.

Irreversible changes that a given temperature regime may bring about in the organism will also be considered under this heading, although there is no suggestion that such changes are necessarily due to the influence of any identity other than temperature so that perhaps they should more properly be considered as the effects of acclimation during a certain critical period, presumably during development (cf., 52). The magnitude of such early effects may be substantial. Gibson (53) found that the temperature at which the early development of the guppy takes place has an effect on the lethal temperature which is roughly equivalent to that of the final thermal acclimation. A similar irreversible effect of the rearing temperature has also been found in *Drosophila* (28).

With regard to acclimatization shown by a measurement of activity, Dehnel (54) showed for three species that the growth of gastropod larvae, subsisting on their yolk, was more rapid at all temperatures up to the local summer maximum (14°C.) in Alaskan populations than it was in the same species in California up to the maximum there (20°C.).³

Adaptation.—Heuts (55) has presented further evidence of genetic differences in the relation of the development of the egg to temperature and salinity in races of the two-spined stickleback. Thermal races have also been reported for a Japanese loach (56) and for a hymenopteran (57). Differences in cold hardiness have been demonstrated for three varieties of alfalfa (58). Temperature sensitivity in a strain of *Paramoecium* has been found to be due to a single recessive gene (59). Pantyukov (60) found that samples of the eggs and pupae of two insects from the Volga territory were cold-hardier than samples from the North Caucasus but it is not clear whether these are more than acclimatization differences.

With regard to species differences, Scholander *et al.* (61) have provided

³ Without transplantation and rearing experiments it is impossible to separate cases of acclimatization and adaptation when dealing with samples of what are otherwise considered to be one species. Hence, it is conservative to ascribe the total effect that Dehnel found to acclimatization.

the largest series of comparisons of the resting metabolism in relation to temperature yet available for arctic and tropical fishes and aquatic invertebrates. It was their conclusion that the standard metabolic rates of the arctic species showed essentially the same response as those of the tropical ones except that the curves were shifted markedly to the left so that they reached their maxima in the region of 20°C. whereas the maxima for the tropical species came at about 40°C. With the ordinary diminution of the Q_{10} that the above curves show with increasing temperature, this means that the arctic species were in relatively the same region of temperature response from 5° to 15°C. that the tropical ones were in from 20° to 35°.

In contrast to the extensive lateral shifts found in the curves of standard metabolism of the aquatic forms, Scholander *et al.* found little difference in the response of the metabolic rates of various terrestrial invertebrates from the two extreme environments. Nor did these workers (62) find any substantial differences arising from similar measurements of the respiration of various tropical and arctic lichens. These results are what would be expected if the adaptations of the terrestrial poikilotherms were largely related to resistance to the annual temperature minimum.

Adaptive differences in the tissues and cells of poikilotherms that would be related to their temperature tolerance have not apparently been extensively sought. Musacchia *et al.* (63) conclude that generalisations concerning tissue composition in fish and adaptability to arctic conditions cannot be made at present.

Mechanisms for thermal adjustment.—The possibilities for thermal adjustment lie within the cell itself, at the cell surface and within the various anatomic systems of the organism. Evidence for adjustment has been sought at all three levels. Some of the best evidence for autonomous adjustment comes from the local differences in response to temperature changes in the exposed parts of homoiotherms, such as the gradient of nervous conductivity observed in the metatarsal nerve of the cold-acclimated gull (64) and other localised responses (e.g., 65). Differences in the respiration of tissue slices in relation to temperature when taken from animals of different thermal history (39) possibly also indicate an extensive degree of independent tissue response although, since these occur also in the deep tissues of homoiotherms (cited below) which are not likely to have any direct contact with the environment, the evidence is not conclusive. Ushakov (66), who found no differences in the rates of dying of various tissues from molluscs and leeches from different localities may not have been justified in concluding from this evidence that racial differences were confined to systemic adjustments. Since he apparently brought all his material to one laboratory for comparison he may have maintained his specimens all under the same conditions and inadvertently brought about a common acclimation before his experiments were carried out.

The most extensively developed hypothesis to account for cellular changes in thermal adjustment is that of Christophersen & Precht (14, 67,

68) who have extended the idea that the viscosity of protoplasm limits the metabolic rate. They consider that bound water held by the protein molecule, which consists in part of water held by the dipolar charges of the molecular chain, part held by capillarity in the intramolecular spaces and part held by the Donnan equilibrium, is to a greater or lesser degree, according to its disposition, impermeable to solutes. The relation of the nonsolute-bearing space to the solute-bearing space (the free water and the bound water held by the Donnan equilibrium) governs the ease with which the metabolites can be mobilised, and thus the metabolic rate. The absolute amount of bound water within a cell is taken to govern the stability of the protein (enzyme) molecule. The stability of the molecule governs its ability to resist temperature extremes. Heat denaturation is looked on by them as a breakdown of the micellar structure, not the extreme of the coagulation of the protein. Cold death is considered to be a withdrawal of vital water from the cell (69, 70). Flexibility in response is provided by a certain specificity of behaviour of molecules of a given protein.

There is considerable evidence in support of this hypothesis. Adjustment which produces increased resistance is thus readily explained by the reduction of free water. Such an effect has often been brought about by simple drying, as for example (71) has been found for the eggs of the brine shrimp. Increased thermal resistance in the active stages of insects has been found to be associated with conditions that induce desiccation (23) and a concentration of the haemolymph (26, 72). Christophersen & Precht (73) produced a slight increase in the cold-hardiness of yeast cells and their dehydrogenases as a result of acclimation at a higher temperature. Convincing parallels were demonstrated between the effects of salts on the hydration of gelatin and on the activity of enzymes (74). The shifts in the metabolic rate with acclimation have been related to changes in tissue water content and enzyme activity by Precht and a number of his students, the results of Stangenberg (75) being typical. She found a greater amount of free and a lesser amount of bound water in muscle from frogs acclimated to 6°C. as opposed to 20°C., together with appropriate responses in the metabolic rate and enzyme activity. A higher water content has also been found in the cold-acclimated bullfrog (76).

However, cold-hardiness of insects is not simply related to their moisture content (77) nor have all determinations of tissue water shown an increased hydration in cold-adjusted poikilotherms. In particular the water content of the brain of the toadfish is notably lower when cold acclimatised than when warm acclimatised but the metabolic rate of brain from the cold-acclimatized animal shows the higher metabolic rate as usual at a given temperature (78, 79). The total water content of goldfish was found greater on cold acclimation, even on a fat-free basis (80).

Perhaps the greatest weakness in the hydration hypothesis is its ability to explain those phenomena which, with current analytical methods for the determination of water, make it difficult to obtain clearcut proof of its

validity. For example, on the basis of the behaviour of the minor fraction of the liver dehydrase which survived at the higher test temperatures it is concluded that the goldfish loses thermal resistance at high acclimation temperatures (36). Since this conclusion obviously does not apply to the intact fish (51), the discrepancy is accounted for by assuming that heat death is due to a systemic breakdown. On the other hand since the goldfish becomes less cold-resistant with increasing acclimation temperature it is concluded that cold death is a cellular death. While these conclusions may be true, they certainly have not been proven. Indeed the general weight of evidence (49, 81, 82, 83) at present indicates that cold death of fish is often a systemic death probably caused by the suppression of the metabolic rate to the point where there is not enough energy available for the maintenance of osmotic balance. There is considerable indication too that heat death is likely to be due to the breakdown of certain critical cells (19), the most telling evidence for this being the general lack of any relation of size to order of heat death (e.g., 46).

The hypothesis of Christophersen & Precht, as they emphasize by their distinction between resistance adjustment and performance adjustment, points out an essential conflict between the ability of the cell to be active and its ability to resist temperature extremes. For the cell to be active it must have a high degree of irritability. For the cell to be able thus to respond to its best degree its standard metabolic rate must be high. At least this is implicit in the literature since the time of Krogh (84) although there are few direct comparisons between the level of standard metabolism and the level to which the metabolic rate can be further raised by appropriate stimulus. High performance therefore requires a high lability of the active constituents of the cell. High resistance requires a high stability of these key cellular constituents and a relatively low rate of metabolism. The aquatic animals which show a high level of performance at low temperatures have a low resistance to upper lethal temperatures and to various poisons, as Schlieper and his students have emphasised (85 to 89). They probably also have a low resistance to temperatures below freezing. Payne (90) years ago found that aquatic insects showed no cold-hardiness. On the other hand the terrestrial poikilotherms of the extra-tropical regions, which must possess a major degree of cold-hardiness, in general have their metabolic rates reduced at temperatures close to freezing and do not show any notable diminution in their ultimate upper lethal temperatures. It will be of major interest to have much more work done to test thoroughly the hypothesis of Christophersen & Precht and to clarify certain ambiguities that have arisen. The chief ambiguity, and the reason why space is not taken here to reproduce their classification of compensatory responses, is that they do not appear to have yet made a clear distinction between the metabolism which represents the energy required for the back reactions continuously necessary to maintain the integrity of the open system which is the living organism (91) and the metabolism which the system can make available for various activi-

ties. It seems clear from Precht's early exposition (67), that such a separation is intended, and the choice of yeast cells for much of their work was made to avoid the effects of physical activity. However, growing yeast cells still must often exhibit a considerable fraction of activity metabolism. In yeast the metabolism available for growth is particularly associated with the anaerobic metabolism (a personal communication from K. C. Fisher), thus the lack of adjustment in the CO_2 production in relation to temperature (92) may have little bearing on the behaviour of the standard metabolism in growing yeast cultures. Approximately 50 per cent of the total metabolism of growing unicellular organisms and developing eggs has been associated with growth and differentiation (93 to 97). The limit for activity metabolism is not necessarily imposed by the same circumstances as impose the limits of standard metabolism and the two fractions should be carefully isolated in critical experiments designed to examine such a fundamental as the adjustment of the cell in relation to its thermal history.

In addition to changes in the water relations within the cell, changes in the nature of the fats have been considered (98). No clear relation has been demonstrated between the nature of the fats and thermal resistance. Hoar & Cottle (99) reported that the iodine number of the body fats of goldfish increased by 0.5 per degree centigrade decrease of acclimation temperature, but some such responses are highly complicated (cf., 100). Natural fats fed to goldfish (101) have a direct relation between the melting point of the fat fed and the resistance to high temperature but no relation to resistance to low temperature was found. When hydrogenated oils were fed, the harder the fat the less was the resistance to both high and low temperatures (99). These authors believe together with Jansky (100) that the number of double bonds may be of importance, rather than the melting point.

No definitive role has yet been found for the endocrine system in the process of thermal adjustment. The response to short term chilling in the turtle has been found to be essentially the same as for such chilling in the mammal (102). Such a short term response is probably not an adjustment however. The thyroid has received considerable attention with conflicting results. Suhrmann (36) found that administration of thiourea to the goldfish obliterated the acclimation response he otherwise found for the whole animal, and the metabolic rate of treated warm-acclimated animals was as high as that of untreated cold-acclimated ones. On the other hand the respiration of tissue slices of treated animals was lower than that of untreated ones acclimated to the same temperature, a result more to be expected. He explains the discrepancy by reference to a finding of Hoar that fish treated with thiourea were more sensitive to stimuli. Hoar found (49) that treatment with both thiourea and thyroxin increased the resistance of summer goldfish to low temperature but neither had any effect on the resistance of winter fish. Oliverau (103, 104) reviews the changes of the endocrine systems of the poikilotherms in general in relation to acclimation temperature.

Fontaine (105) has recently demonstrated that the thyrotropic hormone from a poikilotherm acclimated to a low temperature is much more efficacious than mammalian hormone.

Systemic changes have been but little investigated, except for the endocrine system. Hart (106) has shown a substantial reduction in the blood pressure of summer fish in relation to the rate of heartbeat and an increase in the stroke output. These changes parallel the adaptive differences between species that he earlier described (107). A slight but compensatory shift in the blood dissociation curve of the frog (108) has been found. This change is probably to be accounted for by the change in the blood's alkali reserve. Seasonal changes in red cell volume have been observed (110), some of this change no doubt compensating for temperature change. The data of Cordier & Worbe (111) indicate a slight gain of permeability in minnows acclimated to low temperatures.

REGULATIONS

Behavioural regulation.—The almost universal presence of a faculty of temperature selection in animals has already been mentioned [for a recent review see ref. (2)]. There is an interesting relation between the thermal preferendum and the metabolic rate, first pointed out by Herter (112). If the routine metabolism or the respiratory rate of a poikilotherm is measured over a wide range of temperature, a double sigmoid curve has frequently been found with a central interval, often extensive, over which the rate is little affected by increasing temperature [(43), (113), see p. (36); (114), (115), (116)]. Sometimes there may be an actual drop in the respiratory rate (112). The beginning of the stable interval is related to the thermal preferendum found at a low acclimation temperature (115). The phenomenon is apparently primitively due to a thermal kinesis as has been shown for fish [ref. (7), Fig. 23], although not specifically so reported for amphibia (116) or reptiles (112). Thermokinesis also appears to offer the explanation for some of the changes in the metabolic rate in acute experiments such as Schlieper's findings for the swordtail (117) and may conceivably be the explanation for the unusual results of Berg (118) although he considers his gastropods to be sluggish. Precht (119), who obtained similar results with summer snails, emphasises in his summary that his animals were moving during the tests.

The Saint Girons (120) have provided a recent discussion and review of behavioural regulation in the reptiles with excellent illustrations and an extensive bibliography. The well-known postural responses of insects and preflight muscular activity need no citation here. Viaud (2) has proposed a new term "thermocie" for the communal temperature control exercised by the social insects.

Behavioural regulation is not confined to the poikilotherms by any means. The choice or manufacture of habitat is one of the most effective adaptations in promoting the success of many homoiotherms. Specific differences in behaviour are frequently the most striking variables to be found between

related species to explain their successes in their respective habitats (e.g., 121). The success of the mouse at low temperatures (122, 123) is basically due to its behaviour in building its nest better in that more stringent environment than at room temperature.

Physiological regulation.—The excellent reviews on the homiotherms, particularly with reference to low temperatures, produced by various authorities in the field in recent years (124 to 134; and others given specific reference in the text), make the inclusion of any extensive remarks here an impertinence.

Cold.—All recent work on homiotherms has confirmed the response found earlier to exposures of from one to several weeks at moderately low temperatures whereby there is an increase in the resistance time on exposure to extreme low temperatures, an improvement in the ability to be active at low temperatures (135) and an inhibition of restraint hypothermia (136, 137). There is also an improvement in the animal's ability to warm exposed organs (138). General hypothermia is the most effective stimulus to bring about acclimation to the cold (139). Hart (140) has provided the most recent review on the metabolic response of homiotherms to cold and the relevant remarks below are largely based on this review which should also be consulted for references.

Acclimation of homiotherms to low temperatures is accomplished primarily by the enhancement of their ability to maintain a high metabolic rate. The cost of thermogenesis is added to the cost of work at all temperatures (141, 142). The resting metabolic rate has ordinarily been found to be higher at all temperatures in cold-acclimated subjects than in warm-acclimated ones (140), so that the "peak metabolic effort" (143) is also greater in cold-acclimated homiotherms than in warm-acclimated ones. Cold-acclimatized regulators, in contrast to cold-acclimated ones, in general show a resting metabolic rate at a given temperature which is equal to or lower than that of warm-acclimated ones (140, 144). With respect to adaptation, progress is still based on the firm foundation laid for our concepts by Irving & Scholander and their colleagues (145) who demonstrated the essential uniformity of the basal metabolic rates and body temperatures of the homiotherms of a given size the world over (146 to 151).

Heat.—There appears to be little to add to the summaries of Adolph *et al.* and Robinson (131, 152) regarding compensation of homiotherms to heat. Hellou *et al.* (153) compared acclimation of a group of men in Oxford in a warm chamber to a group acclimatized in the tropics and found a better response in the tropical group. Findlay (154) reviewed the literature for domestic animals pointing out that different breeds of cattle showed genetic differences in their response to an environmental heat load.

Compensatory mechanisms in thermal regulation.—It is well established that much of the thermogenesis in cold-acclimated animals is not associated with shivering or the action currents which accompany it (155 to 160). The disappearance of the action currents led to the suggestion that thermogenesis

on acclimation to the cold is largely of visceral origin and it has been shown that the metabolic rate of various visceral tissues is increased as well as that of muscle (cf., 161, 162). However, Heroux & St. Pierre (163) believe that the red-fibred muscles are more likely to be the most important site of heat production since the capillarity of these is increased by some 60 per cent in rats exposed to 6°C. for four weeks as compared to controls at 30°C. On the other hand, they found no change in the capillarity of the liver. Weiss & Moss (164) found that rats could acclimate to the cold despite sub-total hepatectomy.

The role of the endocrines in cold acclimation has been reviewed in recent years by Dugal (165), Fontaine & Lachiver (166), and Desmarais (167). The thyroid has a slow action in promoting the growth of feathers and fur. It is always important on first exposure to the cold and probably continues to be hyperactive even after long exposure although not to as great an extent as initially (168 to 172). Hsieh & Carlson (173) found that rats acclimated at 5°C. continued to maintain their high metabolic rate after thyroidectomy but reduced their food intake and lost weight. They suggest that this is the mechanism of terminal failure of control in the absence of thyroid. With regard to the function of the adrenals in cold acclimation it has been suggested that the initial activity to meet the first cold stress (174), decreases after about 20 days exposure (175, 176), no more adrenal cortical extract being then needed to maintain adrenalectomised rats in the cold than others on the warm (177). Sealander (178) observed a tendency for the adrenals of rats exposed to 2°C. to return to normal size and activity in about four weeks. Other workers (e.g., 179, 180) have found no reduction of the original hypertrophy on first exposure, but the persistence of large size may not be indicative of continued hyperfunction (181). Desmarais has summed up the work to date on the effects of ascorbic acid on cold acclimation (167). He has come to the conclusion that the primary effect is in conjunction with the thyroid and stresses the interrelation between the thyroid and the adrenals in effecting thermogenesis.

The beneficial effect of high fat diets has recently been reviewed by Pagé (182) who concludes that the sparing action of fats on food utilisation increases in importance in proportion to the increase in total caloric intake and that fats are the primary fuel for extra heat production in the cold. However, Görner (183) could not find any clear increase in the amount of fat consumed in the cold by mice when given their choice of diet. It is not certain that there is a higher degree of lipogenesis in cold-acclimated animals at least so far as tissues other than the liver are concerned.

The early changes in haemoconcentration and water content associated with exposure to cold (e.g., 184, 185) are apparently also only short term responses. Rats, fully acclimated to the cold show no final change in water content on a fat free basis (186) and white mice had a greater water content (187).

The more complete compensations of acclimatisation have a sparing

action on metabolism which is brought about by insulative changes. These changes appear to be of two types, a structural change in the pelt or plumage and an adjustment of the exposed peripheral tissues to an existence at a lower temperature. Hart (140) in summarising the literature, concluded that the overall insulation of cold-acclimated birds and furbearers was only about 75 per cent of that of warm-acclimated ones. He believes that this change is due to the need to maintain the temperature of the peripheral tissues in the face of no substantial change in the insulation provided by the feathers or fur, although slight increases have been found which he summarises. In contrast to the slight effect of cold alone on insulation, the march of the seasons leads to the winter pelts of mammals having an insulation some 10 per cent to 50 per cent higher than do summer ones. The decreasing photoperiod is presumably the prime agent that brings about the increase in pelt thickness (188). The change in the overall insulation may be greater than the change in the pelt alone, as is particularly illustrated by observations on the pig in Alaska (189, 190). This latter change is largely brought about by the adjustment of the peripheral tissues to live at a temperature approaching 0°C. and thus reducing the gradient for heat loss from skin to air. A similar but slighter effect has also been reported for man (144). It is possible that increases in subcutaneous fat also provide a significant increase in insulation. As indicated by the skin temperature, men with a fat content equivalent to 20 per cent of their body weight are approximately 30 per cent better insulated than men with fat equal to only 2 per cent of the body weight (191). In small mammals and birds (150, 192) the possibilities for increasing insulation are limited in terms of greatly reducing the critical temperature.

In homoiotherms fully adapted to the cold the adaptation is almost entirely structural and resides largely in the insulation of the body. Scholander (193) denies that the general form of the animal has any great adaptive significance and touched off an amusing controversy (194, 195, 196) regarding adaptation of the Eskimo; however Allen's rule as applied to ears would still appear of value to an arctic fox. One of the most interesting adaptations is the high development of the vascular heat exchanger in the fins of the marine mammals (197). In terrestrial mammals and man there appears to be a possibility for a variable exploitation of vascular heat exchange (198, 199).

Acclimatization to heat, which has been chiefly studied in man, still presents the same problem regarding enhanced adrenocortical activity as exists with regard to acclimation to the cold (125). There is an increase in body water but little of the final change is in the blood volume (200) regarding which it must be considered that in sweating, man can turn over as much as 70 per cent per hour of water in his blood (201). Practically all heat dissipation at high temperatures is by evaporation and workers acclimated to heat, sweat more freely (202); thyroxine has little effect in reducing the heat load (201). The antidiuretic response to a standard heat stimulus

is some seven times greater in summer than in winter in sheep and man (203) and there are seasonal changes in sweating (204). The camel conserves water by taking advantage of night cooling to reduce the temperature of much of its tissue (205).

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COMPARATIVE PHYSIOLOGY (EXCRETION)¹

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INTRODUCTION

This review is to initiate a series to appear at relatively long intervals, hence a brief statement of objectives seems worthwhile. Without questioning their value to comparative physiology, but in order to prevent duplication, most of the studies of excretion in man and in those common laboratory animals like the rat, cat, dog, and monkey, will be left for the review on kidney function. Emphasis is to be on the work of the last five years but recognition of earlier work will be made when it is necessary to clarify the present knowledge of the field. There has been little activity in certain areas of investigation. In von Buddenbrock's recent Comparative Physiology series (1) the chapter dealing with water and salt economy in protozoa cites three titles for the decade to 1909, four to 1919, twenty-six to 1929, thirty to 1939, four to 1949 and two for the present decade. Though the development of the topic will follow a phyletic approach there will be frequent comparisons with animals of quite other groups wherever it seems profitable. In the hope that more students of renal physiology may be led to undertake comparative studies the writer has chosen to present some controversial matters in a challenging, if arbitrary, fashion. Specialists in invertebrate zoology will recognize that the many errors of omission in dealing with morphological matters were forced by the great complexity of the many important groups of animals with which this review was supposed to deal.

Because of the great current interest in the problems of active transport, osmotic and ionic regulation were reviewed on a comparative basis by Beadle for the 1957 *Annual Review of Physiology*. It has still seemed desirable to touch on a few of the many topics covered by Beadle's review. Other treatments of excretion from a comparative point of view were given by Carter (2), Martin (3), Prosser *et al.* (4) and Schlieper (5).

PROTISTA

For a brief statement of the phyla usually included in this group see Lwoff (6). Though not generally accepted this controversial classification of a third kingdom brings together the protozoa and sponges, the only two animal groups having in common contractile vacuoles and the algae and fungi, the zoospores and gametes of which may have contractile vacuoles [Lloyd (7)]. Its use may therefore serve to emphasize that here we encounter what may perhaps be a major physiological dichotomy. Only a few, perhaps no, animal cells can any longer be thought to play no active part in regulating their osmotic relationship to the environment. This is admirably discussed by

¹ The survey of literature pertaining to this review was completed in July, 1957.

Ramsay (8) and the statement will be left undefended here. As a corollary let us also accept that the osmoregulatory function is the chief role of the contractile vacuole [cf. Kitching (9, 10), and Beadle (11)]. The dichotomy, then, would contrast cells which meet their osmotic problem by actively excreting water by means of contractile vacuoles, with all other cells most of which meet their osmotic problem by equalizing their osmotic pressure with that of the aqueous environment. Since each living cell accumulates essential organic compounds in solution it is not enough to allow the salts of the environment reasonably free access to the interior of the cell, the entrance of equilibrium amounts of all the ions would still leave the entire cell osmotically superior to the environment. The cell therefore actively excretes one or more of the ions and thus maintains its osmotic equilibrium without the necessity of excreting water. Perhaps the dichotomy represents an over simplification; there appear to be protozoa [Kitching (12)] and sponges [Jepps (13)] without contractile vacuoles, and it is possible that the further study of pinocytosis will reveal that the difference in the contrasted groups is one of scale and that many cells excrete minute droplets of water. Declaring it speculative Robinson (14) develops this point of water excretion in a persuasive fashion.

The contractile vacuole may therefore be only an outward manifestation of a protoplasmic or membrane phenomenon common throughout the animal kingdom. Osmotic work must be done either to secrete water into the vacuolar system or the cell membrane must absorb salts from the external medium as rapidly as they are lost by the action of the contractile vacuole. Computations of Lovtrup & Pigon (15) illustrate with measurements made on an amoeba the energy required to accomplish either task, and supplement the values computed earlier by Kitching (16). The amount of energy available is quite adequate for the task but we are not yet able to make a choice between the alternatives although Kitching's (17) demonstration of how quickly *Zoothamnion* pumps out excess water renders the first alternative the more attractive one.

The fluid of the contractile vacuole has not yet been collected and analyzed for its ionic composition. Thirty years ago Weatherby (18, 19) injected xanthidrol reagent into contractile vacuoles of paramecium and collected fluid from vacuoles of spirostomum for urea and ammonia tests. He concluded that no more than one per cent of the urea formed per day was excreted via the contractile vacuoles, the remainder simply diffused out of the cell.

It appears unlikely that foreign substances are accumulated in the contractile vacuole. Howland & Pollack (20) reported that injected picric acid was excreted by the contractile vacuole. The vacuole became an intense yellow then faded progressively as the picric acid was pumped out of the cell. But the estimates of relative concentration were not adequate to prove the case and this interesting observation should be extended.

Koehring (21) kept various protozoa in dilutions of neutral red ranging from 1:1,000,000 to 1:15,000,000 for long periods of time. She described the

accumulation of the dye in the food vacuoles and its loss both by the emptying of food vacuoles and diffusion of the dye back into the protoplasm, but the dye was not accumulated in the contractile vacuoles. Goldacre & Lorch (22) have watched the accumulation of neutral red at the posterior end of a moving *Amoeba discoides*. If the amoeba was not in motion uptake was much delayed and not specific for the tail region. The ultimate fate of the collected dye was not followed but these observations served as the basis of a most interesting theory of a mechanism [Goldacre (23)] for accumulation of various materials. Goldacre argues that a folded protein, characteristic of the sol state of protoplasm, has the active groups of its many side chains coordinated with similar active groups across the fold. When the sol has been moved forward through the interior of the amoeba and to the exterior to become cortex and the protein is unfolded into the gel state these active groups are unsatisfied and will pick up whatever charged material is available in the medium. If the protein moves to the rear and folds again it will transport these adsorbed molecules to the point of folding where they will be given off (accumulated). The accumulation of dye in the tail would therefore be explained and according to the observations could only take place when the cell moved. Whatever factors produce folding and unfolding and movement of the protein produce the pumping action, but of course a stationary protein down which waves of folding and unfolding progress would also serve as a pump. The specificity would be conferred by the nature of the active side groups. This ingenious hypothesis has received wide attention though the observations do not correspond with the descriptions of dye accumulation given by cytologists, like Koehring (21) and Hopkins (24) who attributed the accumulation of the dye in food vacuoles to reaction with proteolytic enzymes. The two approaches are hardly comparable, however, since the cytologists have worked over long periods of time with solutions of neutral red at about 1:1,000,000 dilution, while Goldacre & Lorch (22) showed a high degree of accumulation in the tail after fifteen minutes in about 1:60,000 solution, an amount which is toxic to the animal after a few hours. In neither case were the contractile vacuoles involved.

Sponges are organized at such a level that there is no common excretory system and each cell of a fresh-water sponge maintains its own osmotic balance. After a long debate the fact that cells of fresh-water sponges do possess contractile vacuoles has been excellently confirmed by Jepps (13) who describes them in both amoebocytes and choanocytes of *Spongilla* and *Ephydatia*. It seems reasonable to assume that they are also present in the small cells that comprise the epithelium, the pinacocytes, since the author says "... unless they were being sought the latter (contractile vacuoles) especially could quite easily be overlooked." Though she failed to find them in several marine sponges she notes that they have been figured there by older authorities. Another careful search for such structures might be rewarding, perhaps assisted by acclimatization to somewhat diluted sea water for a period of time. If contractile vacuoles represent the means of obtaining

osmotic equilibrium throughout the whole sponge group then it should be possible to demonstrate a low-level activity of contractile vacuoles in marine forms just as has been done in some of the marine protozoa [cf. Lloyd (7) and Kitching (25)].

The archeocytes of sponges show another excretory function which may be limited to undigestible residues or which may be important in ridding other sponge cells of soluble waste products as well. Van Weel (26) has described the process whereby an archeocyte takes over an excretory vacuole from a structural cell of the sponge, usually a choanocyte, and transports it to an excurrent channel. He points out also that phagocytosis goes only only in incurrent channels, never in excurrent channels. Van Weel believes there must be some orienting factor which enables the wandering cells to find the excurrent channel so efficiently and remarks that this remains an interesting unsolved problem. Kilian (27) verifies the observations but has two objections to van Weel's view. First, he says, the anatomy of the sponge is such that the chances are about ten to one that the archeocyte would find an excurrent channel if it took the shortest route from behind the choanocyte to the nearest water channel; and second, that because of the digestive and respiratory processes going on new substances may be added to the water, or even ionic changes could take place in the flagellated chambers thus giving a polarity which could guide the archeocytes. The suggestions are interesting but in the absence of experimental evidence van Weel's point seems well taken.

COELENTERATA

The gastrodermal cells of this group of animals phagocytize food particles and become highly vacuolated; in this respect they resemble the protozoa to a marked extent. But contractile vacuoles apparently are absent. The cells do not separate as well as sponge cells, making the search difficult, but it may tentatively be concluded that this group of animals belongs to the higher group of marine animals the cells of which adjust their osmotic pressure to match that of the sea water by pumping out certain ions. As long ago as 1903 Macallum (28) demonstrated the accumulation of potassium intracellularly in *Aurelia aurita* and *Cyanea*, a process which occurred simultaneously with a reduction in the cell concentration of Mg and Na. Macallum's results were essentially confirmed by Koizumi & Hosoi (29) with *Aequorea*, *Dactylometra* and *Cyanea*. Robertson (30) wished to avoid the problem of cell components as far as possible and so used the jelly of *A. aurita* from which he had sliced the cells with a razor blade. He used the valuable technique of analyzing before and after dialyzing samples against the environmental sea water to exclude the effect of a simple Donnan membrane equilibrium in each case. Even after this much-neglected control he found all ions differed from sea water. Sulphate was low but potassium was not as high as was found for the whole animals by the earlier workers. Values of several of the ions were in the opposite direction from that expected of a Donnan equilibrium. It is clear that the ionic composition of the jelly must

be controlled by the active intervention of the delicate boundary layer of cells.

The marine coelenterates actively regulate their ionic concentration but apparently have no great problem of osmotic balance since they remain in over-all equilibrium with the sea water. Fresh water coelenterates have not been carefully studied until the work of Lilly (31). She showed that the cells are permeable to water and to the common ions, that the cells contain more solutes than the environment and that the cells actively accumulate Na, K and Br. It is inescapably clear that the cells must somehow pump out water, and if not by contractile vacuoles then by some less conspicuous mechanism.

This group of animals is carnivorous and it is known that nitrogen is lost in several different compounds (Prosser *et al.* 4), but apparently without special mechanisms for transport.

EXCRETION BY NEPHRIDIUM

Excepting the phylum Mesozoa the animal groups at a stage of evolution beyond the radiata up to and including the annelid worms (and even Amphioxus) have evolved a special structure for excretion, the nephridium. Instead of each cell having to meet the problem of water excretion there is a steady elaboration of impermeable outer membranes and a *milieu interieur* guarded by specialized excretory structures. The animals are so diverse that the following brief statements do not cover every case. Details of comparative morphology may be found in the series of recent texts by Hyman (32). Only two chief types of excretory organ will be described in a few words, the protonephridium and the metanephridium.

The animals without any coelomic cavity (acoelomates) and those with a persistent blastocoel (pseudocoelomates), thus including such important groups as the flatworms and the nematodes, usually have a protonephridium with one or more flame bulbs. A single uni- or multinucleate cell within a cup formed of its own protoplasm gives rise to a tuft of cilia, the so-called flame. The cilia presumably set up a current in a tube begun by the same single cell but continued by the interposition of several or many other cells before the exterior is reached through a nephridiopore. The flame cell shares the general property of maintaining the integrity of its own cell contents, but must possess a different polarity from the other cells. Its secretion would pass into the intracellular tube in which the flame plays, next into the intercellular tube, and so to the exterior. But [following Carter (2)] since the cup in which the flame lies is closed at the source of the urine one has to assume either that the flame is giving rise to a filtration pressure, or that it is necessary for mixing of a secreted fluid, or that it has no known function. Of these alternatives the first is the most attractive. After a review of the widespread occurrence of filtration as an initial process in urine formation in the kidneys of animals of the higher phyla Martin (3) pointed out the evolutionary significance of an initial process of filtration in protonephridia which might have as its source of pressure the action of the flame as suggested most recently by Bahl (33) and Pantin (34). But of more immediate importance is

the possibility that modern methods of analysis might resolve the question of whether or not filtration is taking place without the necessity of measuring the pressures involved in so small a system. If filtration takes place in the flame bulb protonephridium it must be through the cell-thick layer of protoplasm, but there would then be an opportunity for reabsorption in the intercellular part of the tube that intervenes before the filtrate reaches the nephridiopore.

In judging the possible importance of a process of filtration in protonephridia it would be useful to have some knowledge of the quantities of fluid produced in some of the animals possessing them. Fortunately good estimates are available from studies of species of rotifers in which the fluid discharged from the protonephridia enters a small bladder before it is expelled into the cloaca and thence from the body of the animal. The bladder can be seen to distend and contract rhythmically in a fashion reminiscent of the contractile vacuole, and in the same way its volume can be estimated and the frequency of its contraction counted. Lürman (35) has verified earlier estimates of the relative volumes pumped out, has extended the observations to several other species, and has demonstrated that the water is swallowed by the animals since tying off the mouth stops the pumping, but the animals do not swell. The surprising result emerges that in an animal which has a sclero-protein cuticle, shown by the experiments just mentioned to be far less permeable than a cell membrane, a volume of water equal to the total volume of the animal may be excreted in from 12 to 90 min. depending upon the species. It would appear that a good filtering device might be more advantageous for further evolution than a secreting mechanism like that presumed for the protista.

Many secreting and absorbing structures possess much alkaline phosphatase in their cells. Danielli & Pantin (36) applied Gomori's technique to the protonephridia of the nemertine *Geonemertes dendyi* and the planarian *Rhynchodemus terrestris* and demonstrated much alkaline phosphatase in the proximal convoluted canal in each case but there was none in the flame bulbs. This indirect evidence at least fits the picture of a filtrate passing down the duct with a composition changed by the active intervention of cells lining the duct.

The next step in the development of the protonephridium occurs in closely related animals with the conversion of the flame bulb into a solenocyte. The animal groups concerned are the trochophore larvae of annelids and molluscs, and the adults of kinorhyncha, some polychaete worms and *Amphioxus*. But in these cases there is a coelom (in the larvae only a pseudocoelom) in which the solenocyte lies. The cellular tubule is preceded by a very long, very thin-walled intracellular tubule in the whole length of which a single or a few very long cilia beat. This structure because of its thin wall and because it lies much more free in a surrounding fluid seems better adapted to filtration, though such filtration has never been demonstrated. Again if there is a filtered fluid it is exposed to the possibility of tubular secretion and reabsorption.

Finally, in the majority of the annelids and in the other phyla up to the molluscs, metanephridia are encountered. These tubular structures open into the coelomic cavity through a ciliated nephrostome. Filtration of fluid takes place through the lining of the coelom into the coelomic cavity from whence the cilia propel it down the tube, the filtrate being modified by reabsorption and secretion as it passes the cellular portion of the tubule. Bahl (33, 37, 38) has been able to collect urine, coelomic fluid and blood from the earthworm *Pheretima posthuma* and to show that urea is probably simply filtered out of the coelomic fluid and blood. But sodium, potassium, magnesium, calcium, chloride, glucose, protein, creatinine, and phosphate all appear to be reabsorbed completely or in part. More recently Ramsay (39) has applied his very elegant micromethods to the collection and assay of fluid from the nephridial tubule of the earthworm *Lumbricus terrestris*. His analyses show very clearly that the hypotonicity of the urine of this animal is achieved in the wide portion of the tubule.

Roots (40, 41) has investigated some of the water relations of *L. terrestris* and *Allolobophora chlorotica* and demonstrated the increased activity of the nephrostome cilia when there is more water to be disposed of. The regulating factors however are unknown. In two nereid polychaetes Barker-Jorgensen & Dales (42) describe almost complete volume adjustment of *Nereis diversicolor* when transferred from 250 m.eq. Cl/l. to fresh water with only 1 to 2 m.eq. Cl/l. Under the same circumstances *Nereis virens* died in a few hours but did adjust at 67 m.eq. Cl/l. Not enough is known of the nephridial function of these animals to partition the responsibility for water and salt excretion and absorption.

MOLLUSCS

Perhaps it was to be expected that the first applications of modern methods of renal physiology to invertebrate animals would be with the larger members of the higher phyla. There is recent interesting work to report on both molluscs and arthropods.

A brief description of the generalized molluscan excretory system may be abstracted from Goodrich (43). All traces of the nephridial system of the trochophore larva are lost at metamorphosis. The coelom remains the location of the filtering devices which are quite different in the different classes so far studied. The coelom itself is reduced to three remnants, that surrounding the gonads, the pericardial coelom, and the renal coelom. The gonadal coelom may drain through an orifice into either the renal or pericardial coelom, and the pericardial coelom usually drains into the renal coelom through a small reno-pericardial opening. The lumen of the kidney drains to the exterior through a duct that in some classes also serves to carry the sex products to the exterior.

In 1937 Picken demonstrated filtration into the pericardium of *Anodonta cygnea*, a fresh water clam (44). When he opened the pericardial membrane copious quantities of filtrate could be drained off. The fluid was nearly free of protein but otherwise corresponded in composition to the blood. Florkin

& Duchateau (45) confirmed the filtration and reported that it took place through the wall of the ventricle. The filtrate passes next into the kidney proper where it is subjected to reabsorption. Picken showed that salt is conserved, the urine containing significantly less than the blood or pericardial fluid. Florkin & Duchateau found chloride, calcium, and inorganic phosphate were specifically reabsorbed.

The same principles apply to a terrestrial gastropod [Martin (3)]. In the giant African snail, *Achatina fulica*, filtration did not occur into the gonadial or pericardial coelom but apparently from arterial capillaries in the wall of the kidney. Urine collected under low pressure from a well hydrated animal showed an inulin content essentially the same as that of blood, though the protein content of the urine was much lower than that of the blood. The filtrate passing over the trabeculae which break up the lumen of the kidney and greatly increase its internal surface is exposed to reabsorptive forces and glucose and salts are both absorbed here. No specific ions were studied but the freezing point depression of the urine was -0.285 compared to that of the blood which was -0.462 . Not enough glucose is present in either blood or urine of the normal snail to account for freezing point depressions of this magnitude, hence salt reabsorption appears to be responsible. The reabsorption of glucose could be prevented with phlorizin, whereupon the urine/blood ratio became one like that of inulin. The kidney of *Achatina* was also found to be able to secrete *p*-aminohippuric acid and phenol red into the lumen as shown by urine/blood ratios which were much higher than that of inulin studied simultaneously.

Harrison (46) has demonstrated the same phenomena most satisfactorily in a cephalopod, *Octopus hongkongensis*. Previous experimenters have nailed these animals to a board or otherwise restrained them. Her animals were resting quietly, unrestrained, a considerable period after the completion of cannulations of the vascular and excretory systems. She studied the simultaneous excretion of inulin, glucose, *p*-aminohippuric acid, and phenol red. The inulin served as a measure of filtration, since its concentration in the urine remained the same as that established experimentally in the blood. The glucose was filtered and subsequently reabsorbed, a process which was inhibited by phlorizin. *P*-aminohippuric acid and phenol red were increased in the urine to about 40 times and 20 times respectively the concentration in the blood. This active secretion was almost completely inhibited by di-nitrophenol. The comparison to the vertebrate system is therefore a most striking one though the morphology is entirely different. The organs upon which data have been published to date are the so-called renal appendages. About 300 in number they represent diverticula from the walls of the vena cava and if they are indeed the location of the filtration process they would nearly complete a full cycle of the circulatory system for it will be recalled that the heart was the site of filtration in Anodonta, arterial capillaries in *Achatina* and now perhaps the great veins in a cephalopod. For a source of filtration pressure it should be noted that the veins in these organisms show strong peristaltic contractions, which Harrison noted also to extend over the

sac like structures of the renal appendages. No pressure measurements have, so far, been made.

The renal appendages of an octopus represent a much folded single or double layered epithelium, the folds forming a myriad of small apertures through which urine escapes from the deeper folds of the structure. A group of organisms so different from all other known organisms that they have been assigned the status of a separate phylum: the Mesozoa, are known only from this habitat, different species infesting the kidneys of different cephalopods. An interest in rearing these unique animals, which possess the interesting property of having a fixed cell number, has resulted in a recent contribution to our knowledge of the urine of the octopus. Emanuel & Martin (47) have reported details of the inorganic composition of the urine. Since the urine was collected from many different animals no attempt was made to analyze each corresponding blood and sea water for quantitative comparison according to the technique of Robertson (30). Emanuel is in the process of publishing careful identifications of the organic components, some of which appear to be new to the animal kingdom. A first report [Emanuel 48] presented the methods of handling the urine and a composite chromatogram for orientation. The accompanying second report [Emanuel (49)] described a new compound, dicyemin, and presented considerable evidence for a hypothetical two- or three-membered lactone structure. Moreover there is suggested an excretory role in nitrogen transport for the compound which, if shown to be correct and quantitatively important, would be the first highly original contribution to nitrogen excretion for several years. In the last report to have appeared at the time of writing [Emanuel (50)] the presence of hypoxanthine and glycine betaine [cf. von Furth (51), and Hoppe-Seyler & Linneweh (52)] are confirmed and guanine is added as a quantitatively important constituent of this urine.

ARTHROPODA

This phylum is so large that generalizations on excretion must be limited at least to classes. The class Insecta, in part because of its great economic importance, has been reviewed exhaustively and recently enough (53) that it will be omitted here. Relatively little has been done on the excretory physiology of some of the smaller classes. This review will therefore deal primarily with the crustacea.

Referring again to Goodrich (43) we find the origin of the excretory organ of a typical crustacean to be from the primitive coelomoduct. It consists of a first part, a thin-walled sac often held open by strands of connective tissue running to neighboring structures, which seems suitable for filtration. That a source of pressure for filtration may be present has been shown in at least one carefully investigated case for Parry (54) finds an adequate arterial blood supply in *Palaemonetes varians* only to this sac of all the structures of the kidney. The physiological evidence for filtration may be said to have begun with the work of Picken (55) who showed that the hydrostatic pressure of the body fluids exceeded the colloid osmotic pressure in *Carcinus maenas*,

Potamobius fluviatilis and *Peripatopsis sedgwicki*. If we assume that the excretory system is open to the exterior and the end sac is held open by connective tissue it is apparent that filtration can occur, even without the local arterial pressure described by Parry.

The end sac communicates with a canal lined with cuboidal or columnar epithelium where other changes may take place in the urine. Peters (56) as long ago as 1935 demonstrated the urine to be hypotonic to the blood in *P. fluviatilis* and believed this to be due to a process of filtration into the end sac followed by absorption of salt in the convoluted tubule. In 1940 and 1941 Maluf (57, 58, 59) described the excretion of urine by the freshwater crayfish *Cambarus clarkii*. He interpreted his many experiments to show that all the components of the urine were secreted, including the substance inulin, so commonly taken as an indicator of filtration. His interpretation of the hypotonic urine differed from that of Peters, since now the salt secretion was outward, rather than inward and the accompanying water secretion was such that the finished urine would be hypotonic. Arguments for believing that Maluf may have erred in his interpretation have been presented in detail [Martin (3)], with the conclusion that the experiments may instead show filtration into the end sac followed by reabsorption of water, salts, and glucose (xylose was the substance used). The matter is crucial to a proper understanding of excretion in freshwater arthropods and should be resolved by further experimental analysis.

The application of modern methods of renal physiology to the crustacea was not delayed by this difficulty, however. Forster & Zia-Walrath (60) quickly showed that there was no active secretion of inulin in a marine form, *Homarus americanus*. Since that time, working with the same lobster, Burger (61, 62) has confirmed that inulin is filtered and not reabsorbed and that there is not sufficient reabsorption of water to alter a urine/plasma inulin ratio away from one during 28 hr. after a single injection of inulin. Nonprotein nitrogen was also found to be filtered but the blood/urine ratios were variable and it was concluded that there are substances, of which trimethylamine oxide is one, which are excreted by the kidneys. But the urine level of ammonia and urea is so low that it is clear that these substances must be lost primarily through the branchial membranes. Glucose is filtered but reabsorbed, a process which could be poisoned with phlorizin. The blood level of glucose could be artificially raised to 100 mg. per cent without glucose appearing in the urine, but useful tubular maximum values of rate of reabsorption could not be calculated because the flow of urine was extremely variable. A part of the explanation of this variability seems to lie in the variable blood protein levels for there was a positive correlation between the blood protein level and the volume of urine formed per unit time. Burger concludes that water is normally taken up osmotically and excreted by the kidneys. The kidneys are able to concentrate phenol red, *p*-aminohippuric acid and magnesium. The over-all picture is thus very similar to that of the higher molluscs.

But while the marine forms meet their osmotic problems with ease and while some freshwater crustaceans produce a hypotonic urine, another in-

vader of fresh water has failed to use the kidney in this way. Parry (54, 63) has described some very interesting prawns that have invaded brackish water but continue to put out both large volumes of urine and urines that are isosmotic to each animal's blood. In a recent paper (64) aspects of kidney function of a prawn completely adapted to fresh water, *Palaemonetes antennarius*, are described. Although living in fresh water this form excretes urine isosmotic to the blood in a volume estimated at two per cent of the body weight hourly. It is able to absorb salt so efficiently from the soft water environment that it multiplies successfully. This prawn does, however, come close to an important criterion for the successful invasion of fresh water [Beadle & Cragg (65), Potts (66)] namely that of somewhat reducing the blood salt concentration and hence the urine salt concentration from the high levels of its relatives in the sea. In a somewhat similar situation the ability of the crab *Eriocheir sinensis* to absorb salt through the gills, demonstrated so nicely by Krogh (67) for the whole animal, has been studied with the gill *in vitro* by Koch *et al.* (68, 69) in a very elegant series of experiments. The gills work so effectively that there has been no necessity for the development of salt reabsorption. But *Eriocheir* apparently does not produce the large relative volume of urine that *Palaemonetes* does. Parry estimated the rate in the latter to be ten times that of *Cambarus* and *Astacus*. A method of comparing the relative rates of transport has been illustrated by Wikgren (70) in a report in which he draws together some values for different animal species. *Rana esculenta*, transporting $210 \mu\text{M}$ of Cl/100 gm./hr. led the field. Several species of fish were next in rank but considerably lower in rate of transport at from 20 to $90 \mu\text{M}$ of Cl/100 gm./hr. *P. fluviatilis* led the group of invertebrate animals at $20 \mu\text{M}$ /100 gm./hr. Computation from estimates in Parry's paper shows that *P. antennarius* transports chloride at an even faster rate than *Rana* since it can move $256 \mu\text{M}$ /100 gm./hr.

The question still remains open whether or not from this outpouring of fluid there is anything of use to the animal being salvaged by the kidney. Since perhaps glucose is not of direct metabolic importance to these animals [cf. Scheer (71)], the possible reabsorption of some other metabolites like amino acids might be worth the search.

Following the great burst of research on the forms of nitrogen excretion that led to the generalizations of Delaunay, Florkin & Needham in the years from 1920 to 1940 (adequately reviewed by Prosser *et al.* (4) and Florkin (72) there has been only a very slow pursuit of the remaining quantitative problems. A. E. Needham (73) has now kept many individual specimens of *Carcinides maenas*, of about 25 gm. body weight, in 100 ml. of water, changed daily for more than a year. Daily estimations of total nitrogen and several forms of nitrogen were made. He was able to present many conclusions, too numerous to be detailed here, and the method is susceptible of many interesting comparative results.

VERTEBRATES—FISH

The first volume of "The Physiology of Fishes" by Brown includes a chapter on excretion and osmoregulation by Black (74) which considerably

extends her 1951 review of the same subject (75). The appearance of so recent a review makes it possible here to select a few of the more puzzling problems for discussion from a field that has been quite active.

With the intervention of the war years there appeared without note in the *Annual Review of Physiology* the 1943 paper by Kempton (76) denying the presence of a special segment for urea reabsorption in the tubules of elasmobranch fish. The second paper of the series appeared ten years later [Kempton (77)] dealing with the physiology of urea reabsorption by the dogfish tubule. As first postulated by Smith [cf. Smith (78)] and demonstrated by Clarke & Smith (79) it is here confirmed that there may be up to 99.5 per cent reabsorption, depending upon the plasma urea concentration. The site of urea reabsorption could not be localized. Unfortunately no experiments with metabolic inhibitors were reported so that we cannot compare the lability of transport in this direction with that reported for transport to the lumen of the frog renal tubule by Forster (80).

W. W. Smith (81) first noted the fixed acidity of fish urine and argued that it was a necessary adaptation in view of the simultaneous excretion by the fish kidney of phosphate and magnesium in large quantities. The fish obtain the magnesium by drinking quantities of sea water to provide water for excretion. The chloride ion is excreted by the gills but the Mg and phosphate cannot be excreted by this route. The combination of these ions into magnesium phosphate can only be maintained at a pH below 6 or magnesium hydroxide will precipitate out. Even the magnesium phosphate is excreted in supersaturated solution as shown by Pitts (82). Hodler *et al.* (83), have investigated the relationship between this fixed pH and carbonic anhydrase and find, contrary to the results with mammals and with freshwater catfish, that carbonic anhydrase is not involved in the acidification of urine of either of two marine fish: an elasmobranch, the dogfish, and a teleost, the sculpin. Their experiments showed, however, that there is a carbonic anhydrase dependent system involved in the branchial excretion of sodium bicarbonate.

Workers with the fish kidney appear to be making progress toward another notable contribution to our knowledge of excretion, not to mention the importance of the findings to the whole area of active cellular transport. Chambers & Kempton (84) in 1933 sought to answer by *in vitro* methods the problem of tubular transport of phenol red. Earlier work was reviewed and it was shown clearly that transport of phenol red takes place in mesonephric tubules of chick embryos without a blood supply. The uptake of dye by the cells as well as its transportation into the tubule lumen was noted and it was observed that cold inhibits both steps. With other co-workers Chambers (85, 86) soon demonstrated the inhibition of dye transport by cyanide, hydrogen sulfide, and iodoacetate. Attempts to reverse these inhibitions with pyruvate, lactate, succinate, and glycerophosphate were not very satisfactory. It may be seen that the well known effect of acetate upon the poisoned system was nearly discovered.

In 1948 Forster (87) extended these observations to slices and teased tubules from the kidneys of the frog and fish, and even tried slices from representative species of every vertebrate class. The fish tubules separated

so easily that this preparation became the one of choice and was intensively studied by Forster & Taggart (88, 89). In early work the dye was transmitted immediately into the lumen and could not be seen in the cells. The next step came as a result of changing the ionic composition of the medium. Puck *et al.* (90), found that by the omission of potassium from the balanced medium no transport of dye whatever took place, but if potassium were present and calcium absent from the medium, dye moved into the tubule cells but not on into the tubule lumen. The concentration of dye in the cells was then proportional to the potassium concentration in the medium and the process of cellular accumulation could be blocked by cold and by drugs. Thus was demonstrated a two-step system which resembled in some ways one which had been postulated by Shannon as early as 1939 (91). Wasserman *et al.* (92), confirmed these results and pursued the study by increasing the concentrations of dye in order to elicit the phenomenon of "self-inhibition." Efforts to find evidence for a two-step process in mammalian kidney tubules so far have been unsuccessful [Forster & Copenhagen (93)] although Beyer *et al.*, as early as 1950 had extended the observations of *in vitro* luminal accumulation to the mammal (94). In view of the successful results of Chambers with the chick mesonephric tubules it would appear wise to approach the problem from a comparative standpoint and in the light of current knowledge examine carefully the tubules of various species of vertebrates.

Forster (95) has continued his careful analysis of ion excretion by fish kidneys and described the osmotic diuresis that occurs in aglomerular fish. Brull, with a number of different collaborators has investigated the activities of the aglomerular fish kidney, in particular its response to perfusion *in vitro* (96 to 99). In view of the great complications produced by diuresis in captured fish this approach may prove to be a fruitful one.

Scholander *et al.* (100), have discovered an adaptation which may be partly renal, partly branchial in the course of determining why fish do not freeze in the Arctic though supercooled by from 0.7° to 0.8°C. The deep-water forms are truly supercooled since they do not change the freezing point of their blood and tissues far from the normal level for fish. They therefore freeze instantly when brought in contact with ice but since they never come in contact with ice crystals they live safely in the supercooled condition. The surface fish appear to be only slightly supercooled because they increase the body load of ions to double the normal osmolarity, almost matching the environment. The salt balance reached must be an entirely different one from normal but analysis is not yet complete nor have the tissues responsible for the change been determined.

AMPHIBIA, REPTILES, BIRDS AND MAMMALS

A text book of Avian Physiology has appeared [Sturkie (101)]. The chapter on kidneys and urine draws together the scattered anatomical, physiological, and biochemical literature, particularly for domesticated birds, and points up the need for more studies in this field. Schmidt-Nielsen, Jørgensen & Osaki (102) investigated the hypothesis that marine birds drink

sea water to meet their water needs. The investigation showed that this is not the case in the cormorant *Phalacrocorax auritus*, but that on a diet of fish the bird has ample water to meet its needs for excretion and heat loss. Upon loading the bird with salt it was discovered that there is a very effective extrarenal route for excretion of sodium chloride. Nasal structures, apparently primarily the nasal gland, produce a secretion twice as concentrated in sodium chloride as the most concentrated urine the bird can produce. This fluid drips from the internal nares and is shaken from the tip of the beak. Production of the nasal secretion may be stimulated by an osmotic load imposed by the injection of sucrose so that the mechanism is clearly one adapted to meet the osmotic requirements of the animal. Birds that feed on marine invertebrate animals instead of fish would ingest more salt and the authors are continuing their experiments to determine the generality of this phenomenon.

The Schmidt-Nielsens and their co-workers have described in a most interesting fashion some of the inter-related adaptations of the camel to desert life (103 to 106). Of primary concern to us here is the water economy which surprisingly enough does not rest upon an unusually high concentration of solids in the urine. The urine volume is low, unless the water intake is abundant, and evaporative losses are unusually well controlled, particularly since the body temperature is allowed to fluctuate within much wider limits than are set by other mammals. In addition the animal is able to function effectively under conditions of desiccation that are fatal to most mammals.

For those concerned with kidney function the work provides another point of departure. When fed upon diets low in protein the animals showed an extremely low rate of excretion of urea. This fact helps to account for the low volume of urine, since urea makes up so large a part of the solids of most mammals but, in addition, the conservation of nitrogen turns out to be most efficient. As has long been known the addition of urea to the diet of ruminants can meet part of the nitrogen requirement for growth. In a sense the ruminant is a "plankton-feeder." Not only does the host utilize the intermediate products of cellulose metabolism produced by the symbiotic fauna and flora of the rumen but at intervals portions of the artificial lake are filtered and the myriad of organisms digested and their amino acids absorbed. On a low protein diet the excretion of a normal quantity of endogenous nitrogen would therefore be very wasteful but the kidney tubules of the camel reabsorb urea actively and it is returned to the rumen, probably in the saliva, to be used again by the fauna and flora. Even when a large amount of urea was injected into the animal only one-tenth of it could be recovered from the urine.

The addition of the camel, and possibly of all ruminants, to the group of animals which is now known to secrete or reabsorb urea revives again the old question of whether the kidney tubules of any animal are completely indifferent to urea. The substance is absorbed actively by elasmobranch and ruminant kidneys, actively secreted by frog kidneys [cf. Forster (107)], but apparently simply filtered by the kidneys of other mammals with some passive diffusion due entirely to the high concentration finally attained in

the tubules. The most recent retesting for urea secretion appears to be that of Blegen *et al.* (108), in the dog. They cite German and Hungarian workers who claim urea secretion but from their own careful work can find no support for the claim. Judging from the comparative physiology of urea excretion it is likely that this argument will continue for a long time and it will not be surprising if other animals, for particular adaptations, are found either to secrete or absorb urea actively in the kidney tubule.

The availability of Diamox has led to tests of the role of carbonic anhydrase in acid-base balance of several more species of animals. Its role in freshwater fish has already been mentioned. Coulson & Hernandez (109) found the enzyme necessary for chloride absorption from the urine of alligators. Wolbach (110) finds carbonic anhydrase plays an active role in the chicken kidney.

Clark (111) has investigated the accumulation of waste nitrogen in the eggs of the black snake *Coluber c. constrictor*. He finds much the same sequence of events, from ammonotelic to ureotelic to uricotelic excretion, as in the bird egg but with a considerably longer duration of the ureotelic condition. In a few experiments with a placental snake the role of urea was even larger since some of the waste nitrogen could be dissipated by transfer to the mother. Such adaptive changes may also take place in the amphibia for Munro (112) in a selected series of amphibians found a good correlation between the form in which nitrogen excretion takes place and the type of environment. Waste nitrogen is secreted mainly as urea if the adult amphibian environment is terrestrial or predominantly as ammonia nitrogen if the environment is completely aquatic. But during metamorphosis ammonia excretion is replaced by urea excretion only when the terrestrial habit, as well as the terrestrial form, is assumed. Nor is the situation with respect to an adult ureotelic or uricotelic condition simple, as has long been known. Khalil & Haggag (113) at the beginning of one experiment on a specimen of *Testudo leithii* found it to be a typical uricotele, excreting uric acid as the main nitrogenous end metabolite while urea was present only in traces. The following successive samples (time units were unfortunately not given but were said to be long) showed a gradual decrease in uric acid and an increase in urea until the animal became a complete ureotele at the end of the experiment. The authors conclude: "... land tortoises, by factors still under investigation, can be ureoteles, uricoteles, or ureo-uricoteles. . . ."

We may conclude the review with one last example of the clarification that may result from a comparative view of a field. The rabbit, that docile and generally cooperative laboratory animal, has been quite otherwise for the student of renal physiology. From work reported by Forster & Nyboer (114) it becomes clear that the rabbit possesses a reflex like the submergence apnea of diving mammals. It is possible that other burrowing animals may also be found to have this reflex, but this is the first demonstration in an intact laboratory animal of neurogenic regulation of renal function. In essence the reflex makes the experimental animal into a head-heart-lung preparation for the duration of its action and if induced inadvertently by an investigator during an experimental interval the results obtained

for the interval would be completely inexplicable. This observation should be very helpful in clearing up past misunderstandings and preventing new ones.

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HYPOTHALAMUS, ADENOHYPOPHYSIS AND ADRENAL CORTEX^{1,2}

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The conviction that the hypothalamus has a regulatory influence over the adenohipophysis is strengthened by new experimental data, but unfortunately the precise mechanism or mechanisms by which this regulatory control is mediated remains unsettled. We have assumed that there is a "connecting link" between the hypothalamus and adenohipophysis which represents a "final common path" for the great variety of stimuli which induce ACTH release. A logical sequence of developments would call for definition of this "final common path" before proceeding to the delineation of the paths which subserve various categories of stimuli. Work has already been initiated to elucidate the tracts from lower and higher centers of the nervous system which lead into the hypothalamus and terminate on the "final common path." In anticipation of new developments in this field we have devoted a relatively large part of this review to the neuro-anatomical features of the hypothalamus and its connections.

The available evidence justifies the popularity of acceptance of the thesis that the portal venous system carries a neurohumor from the median eminence to the adenohipophysis. However, in the absence of unequivocal data to support the thesis or to establish it as the sole or major mechanism of regulation, we have purposely set out a fairly exhaustive classification of mechanisms regardless of available experimental support.

Finally, we have emphasized those contributions on the nature of the secretion of the adrenal cortex which are based on steroid analyses of adrenal vein blood. Quantitative analysis of adrenal vein steroids represents a most profitable approach to pituitary and extrapituitary regulation of steroid secretion. New insight into fundamental problems of the homeostatic control of electrolyte composition and volume of body fluids will undoubtedly be gained within the next year or two as a result of investigations in this area.

THE HYPOTHALAMUS AND ITS CONNECTIONS

The results of experiments in which the region of the tuber cinereum has been stimulated or destroyed suggest that this region exerts a regulatory influence on the output of tropic hormones from the adenohipophysis. The

¹ The survey of literature pertaining to this review was completed July 15, 1957.

² The following abbreviations are used in this chapter: ADH (antidiuretic hormone); TSH (thyroid stimulating hormone); CRF (corticotropin releasing factor).

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hypothalamus has extensive connections with the cerebral cortex, basal ganglia, rhinencephalon, thalamus, midbrain, and collateral connections with all sensory afferent pathways. The delineation of these neuroanatomical connections forms an important basis for the interpretation of the results of stimulation and ablation experiments.

PATHWAYS IN THE SPINAL CORD AND BRAIN STEM

Adequate peripheral stimulation results in ACTH release. This reaction may be mediated by neural or humoral components or both. It has been tacitly assumed that the neural component is the classical sensory pathway to the brain stem. Within the brain stem there are collateral connections through the reticular formation to the posterior hypothalamus (54, 153). From the posterior hypothalamus the pathway continues in an unknown manner to the adenohypophysis. An ancillary pathway is from the posterior hypothalamus down the spinal cord to the adrenal medulla (15). Epinephrine secretion from the medulla has been demonstrated by McDermott *et al.* (105) to influence ACTH release.

TELENCEPHALIC PATHWAYS TO THE HYPOTHALAMUS

Cortical and striatal formations may mediate the influence of emotional states on hypothalamus and lower brain stem through several pathways: the hippocampal formation through the fornix, the amygdala through the stria terminalis and other shorter amygdalo-hypothalamic connections, the globus pallidus through the pallido-hypothalamic fascicle and the orbital cortex through a partially defined pathway. These projections to the hypothalamus all enter into relationship with the median forebrain bundle which is one of the major associational systems of the hypothalamus (64). A more detailed description of these pathways follows.

Pathways from cerebral cortex to hypothalamus.—The orbital cortex projects to the ventromedial nucleus of the hypothalamus as demonstrated by strychnine neuronography (170) and by the modified fine-fiber silver technique of Bielschowsky (169). Stimulation of the orbital cortex has been shown to exert an inhibitory influence on somato-motor as well as visceromotor functions (89). Other areas of the frontal lobe also project to the hypothalamus (108, 170) and medial forebrain bundle [Auer (6)]. The precruciate and prefrontal cortical areas of cats have been shown by the Nauta-Gygax method to project to the dorsomedial nucleus of the hypothalamus and other areas of the brain stem (6). The cingulate cortex projects to the hippocampus and thence, indirectly, to the hypothalamus and other parts of the brain stem via the fornix. The temporal lobe cortical areas have indirect connections with the hypothalamus by means of fibers to the basal ganglia, diencephalon, and mesencephalon [Whitlock & Nauta (172)]. The influence of these areas of the frontal cortex on ACTH release remains to be investigated.

Another means by which the cortex may influence the posterior hypothalamus involves both a neural and a vascular path. Stimulation of the

anterior sigmoid gyrus of the cat has been shown to result in a release of epinephrine from the adrenal medulla [Ferguson *et al.* (48)] over an efferent pathway which does not involve the hypothalamus, but descends in the internal capsule. The resulting increased concentration of epinephrine in the blood stream facilitates the ascending reticular activation system [Bonvallet *et al.* (19); Dell *et al.* (38)]. Facilitation of this system results in arousal of the cortex which in turn could lead to further secretion of epinephrine and in turn to release of ACTH.

Pathways from rhinencephalon to hypothalamus.—The rhinencephalon has extensive connections with the diencephalon and widespread influence on both the somatic and autonomic nervous systems (22, 56, 89, 127). The hippocampus receives impulses from fibers in the fornix (112) and entorhinal cortex as well as from the cingulate cortex by means of the cingulum and supracallosal stria (89). Adey *et al.* (2) have emphasized that the hippocampus is connected with the hypothalamic and midbrain reticular formation through two routes: a pathway from the hypothalamus through the fornix to the hippocampus, described above, and a pathway from the hippocampus through the entorhinal area and stria medullaris to the midbrain reticular formation. Over the former, impulses from the hypothalamus may pass through the fornix to activate the hippocampus [Green & Arduini (67)]. Over the latter, impulses from the region of the hippocampus and entorhinal cortex may pass to the mid brain reticular formation where they may produce a prolonged inhibition of conduction in central tegmental pathways ascending to the hypothalamus. Consequently, increased activity in the hypothalamus may be self-limiting through the mediation of the hippocampus. This may be the mechanism whereby entorhinal-hippocampal stimulation results in suppression of plasma 17-hydroxycorticosteroids in the monkey [Mason, Nauta & Rosenthal (100)].

Nauta (115) in a detailed fiber study of the fornix has demonstrated hippocampal connections to the medial forebrain bundle, the anterior, intralaminar and midline thalamic nuclei, the lateral preoptic area, the lateral, mammillary and posterior hypothalamic nuclei, and the periventricular system (particularly its arcuate part). Guillery (72, 73) showed that approximately one-third of the premammillary fornix fibers, particularly those from the hippocampus, and perhaps from stria terminalis and septum pellucidum, terminate in the medial, postero-dorsal and lateral parts of the hypothalamus and in the periventricular system. The fornix fibers to the mammillary bodies arise in the dorsal fold of the alveus and terminate in the posterior and lateral parts of the medial mammillary nuclei. The remaining fibers pass on to the mammillary peduncle through the supramammillary commissure.

Basal ganglia connections to hypothalamus.—The basal ganglia of the telencephalon include the corpus striatum, the claustrum and the amygdaloid nuclei. Fibers from the amygdaloid nuclei make direct connection to the nuclei in the middle hypothalamic region and to the medial forebrain bundle via the stria terminalis and other shorter amygdalo-hypothalamic connec-

tions (3, 64, 115). Bard & Rioch (8) showed a pallido-hypothalamic fascicle from the globus pallidus to hypothalamus and the medial forebrain bundle. Ranson & Ranson (131) traced a small bundle of fibers from the ansa lenticularis and fasciculus lenticularis to the ventromedial nucleus of the hypothalamus. Much of the functional role of these connections remains obscure. The limbic system is now accepted as the primary autonomic center of the forebrain (89) and studies indicate the region closely related to the amygdala is concerned with control of behavioral patterns characterized by aggressiveness (173).

PATHWAYS FROM MESENCEPHALON TO HYPOTHALAMUS

There are three commonly accepted ascending fiber paths from the midbrain to the hypothalamus: mammillary peduncle, central gray-periventricular system and the medial forebrain bundle. Application of a recently developed fiber technic (116) has shown that unmyelinated fibers in the mammillary peduncle from the dorsal and deep tegmental nuclei (28) end in the mammillary nuclei [particularly in the lateral mammillary nucleus and the pars medianus and pars medialis of the medial mammillary nucleus (73, 74)]. Work remains to be done in further delineating the connections of these fiber paths.

FIBER BUNDLES WITHIN HYPOTHALAMUS

The most extensive group of associational fibers in the hypothalamus is the medial forebrain bundle. This fiber bundle extends from the preoptic region caudally to the mesencephalon (112). The medial component of the medial forebrain bundle probably arises in the midbrain and ends in the medial septal nucleus. Some of these fibers continue into the hippocampus (74). Guillery (74) also demonstrated that the lateral component of the medial forebrain bundle contains fibers which arise in the premammillary hypothalamus and go to the lateral septal nucleus. The other large associational system in the hypothalamus is the central gray-periventricular system. Fibers in the periventricular system connect hypothalamus with the thalamus and also with the midbrain.

THE HYPOTHALAMUS AND ITS FUNCTIONS

A neuro-anatomical description of the hypothalamus indicates that it is composed of a multitude of nuclei the boundaries of which are in many instances ill-defined. These nuclei have extensive connections not only among themselves but also with the rest of the nervous system. Before considering the problem of regulation of ACTH release by the hypothalamus, it should be emphasized that the complexity of structure of this important area of integration is matched only by the complexity of its functions. The hypothalamus integrates somato-motor and viscero-motor activities which are predominantly of a homeostatic nature. The hypothalamus may influence the following: cardiovascular control including vasodilation (130, 165),

vasoconstriction (154), heart rate (90), blood pressure (61); temperature regulation including piloerection (168), vasodilation (165), shivering (18), sweat secretion (162), and panting (99); visceral motor control including gastrointestinal tone (16), bladder tone (14), and micturition (161); water metabolism (49); appetite (21); sleep-wakefulness (82); respiration (133). The hypothalamus also influences the secretion of endocrines: aldosterone secretion (132), epinephrine secretion (50), and the secretion of pituitary tropic hormones including ACTH. Thus the control of ACTH release is but one of the large number of regulatory functions of the hypothalamus. With so many homeostatic processes localized in so small a volume of tissue, the possibility of making a lesion which would result in selective disruption of any single regulatory function is remote. Adding to this complexity are secondary changes. Thus the interpretation of the results of lesion experiments may be complicated because of a multiplicity of aberrations. For example, a lesion which selectively disrupts thyroid stimulating hormone (TSH) secretion may be expected to bring about a secondary change in the pituitary-adrenal system subsequent to the development of the hypothyroid state.

The relative effectiveness with which the lesion techniques may be applied is a function of the anatomical discreteness of the center under consideration.

LESIONS IN THE HYPOTHALAMUS

Posterior hypothalamus.—Lesions in the posterior hypothalamus (44, 69, 123) and lesions involving both posterior and mid-hypothalamus induce pituitary adrenal inertia (92, 105, 150). These lesions were invariably large enough to include more than one hypothalamic nucleus or tract or both. A destructive lesion in the posterior hypothalamus may include posterior, premammillary, supramammillary, and medial mammillary nuclei and their subdivisions. The nerve fibers in the mammillary peduncle, mammillo-thalamic tract, mammillo-tegmental tract, medial forebrain bundle, fornix, and periventricular system may also be involved. Consequently, connections between the posterior hypothalamus and midbrain, hippocampal formation, anterior thalamic nuclei as well as with rostral parts of the hypothalamus may be severed. In addition to destroying a large number of nuclei and pathways whose functions are incompletely understood, these posterior lesions disrupt a center which we know integrates the activities of the sympathetic nervous system. The impairment of the sympathetic nervous system may be in part responsible for the failure of the pituitary to respond to certain stimuli.

Mid-hypothalamus.—Lesions in the middle hypothalamic region have been shown to result in pituitary-adrenal inertia (5, 55, 58, 85, 87, 93, 101 to 104). Destruction of the median eminence appears to be the most common feature of these lesions. However, in most instances, it is not possible to exclude involvement of one or more of the following structures: periventricular, arcuate, ventromedial, anterior, and suprachiasmatic nuclei, and nerve

fibers in the periventricular system, Ganster's and Meynert's commissures, hypothalamo-hypophyseal tract, and fibers from the fornix to the middle hypothalamic region. Destruction of neurons in this area would sever connections between hippocampus and hypothalamus and eliminate contributions to the hypothalamo-hypophyseal tract from the periventricular system, from the medial forebrain bundle, from the lateral tuberal nucleus, from the anterior hypothalamic area as well as from the supraoptic and paraventricular nuclei (66). Possible impairment of pituitary function as a consequence of damage to its blood supply will be discussed in a later section. The complexity of this area precludes selection of any single structure as being responsible for ACTH release on the basis of ablation studies only.

STIMULATION AND RECORDING OF ELECTRICAL ACTIVITY OF THE HYPOTHALAMUS

Stimulation.—Electrical stimulation of the posterior hypothalamus results in a lymphopenia in the rabbit (69), eosinopenia in the cat (4, 123, 124), increase in adrenal vein blood corticosteroids and alteration of sudanophilic substance in the adrenal cortex of the cat (91) and adrenal ascorbic acid depletion in the rat (43).

Recording.—The injection of epinephrine or activation of the sympathetic-adrenal system by insulin hypoglycemia or hypoxia caused a marked increase in the electrical activity of the posterior hypothalamus (122). An increased concentration of epinephrine in the blood has been shown to have a facilitatory action on the reticular activating system in the posterior hypothalamus and rostral mesencephalon (19, 38). This was confirmed by Nakayama (114), who showed that when larger doses of epinephrine were used, electrical activity in the anterior hypothalamus was also increased; moderate and large doses of histamine induced changes similar to those induced by moderate and large doses of epinephrine, respectively. Reticular formation and sympathetic nervous system activity may be altered by other humoral agents. Administration of Pitressin in a dose which has been shown to induce adrenal cortical activation (102), was followed by spike activity in the posterior hypothalamus, fornix and medial forebrain bundle of cats. The spiking occurred after a short latency and persisted for as long as 35 min. after the injection. Electrical activity was still enhanced long after blood pressure had returned to normal [Sutin & Clemente (156)].

HYPOTHALAMIC ADENOHYPOPHYSEAL LINKS

Although there is general concurrence in assigning to the hypothalamus an important role in the control of the adeno-hypophysis, there is little agreement as to how this control is exerted. The two possible avenues by which the hypothalamus may communicate with the adeno-hypophysis, namely neural and vascular, may be further subdivided into central and peripheral neural connections and into portal and systemic vascular connections. Hypothalamic control of the adeno-hypophysis may be mediated by one or more of these pathways.

NEURAL CONNECTIONS

Harris (76) has summarized anatomical studies on the innervation of the adenohypophysis and concludes that it receives very few, if any nerve fibers. Despite the evidence marshalled by Harris, we feel that the question of the innervation of the adenohypophysis is still unsettled.

Smith (152) has used Ranson's pyridine silver technique to investigate the neural tissue within the adenohypophysis of the ferret. He detects uniformly distributed nonmyelinated fibers of unknown origin in the pars distalis. The fibers are few in number, a fact which "may have been due to incomplete impregnation of the material."

Metuzals (107) describes an "autonomic nervous formation" composed of ganglia, ganglion cells, and nerve fibers extending around and into all parts of the pituitary of the duck. He also describes a "secretomotor end plexus" which forms synapses *en passant* with the individual gland cells of an epithelial cord. If a single nerve fiber makes connection with a number of gland cells as the author implies, then a relatively small number of nerve fibers could adequately innervate the glandular tissue. According to Metuzals, the Bielschowsky-Gros method of impregnation which he used is specific for nervous tissue and does not stain reticular fibers.

We concur with Donovan & Harris (39) that any claims regarding the existence of neural tissue within the pars distalis should be supported by the control procedure of staining sections from normal denervated glands on the same slide. Perhaps the technique of Nauta & Gyax (116), for silver impregnation of degenerating axons would be of value in the solution of this problem.

The experiments of Abrams, Marshall & Thomson (1) which demonstrated that bilateral cervical sympathectomy prevents the onset of estrus in ferrets exposed to artificial light have neither been confirmed nor extended in the past year. Studies on the functional relationship between the peripheral autonomic nervous system and the pituitary seem to be temporarily out of fashion.

VASCULAR CONNECTIONS

Portal Vascular System.—The controversy over the functional significance of the hypothalamic-hypophyseal portal vessels as a link between the hypothalamus and the adenohypophysis continues. Harris (76) summarizes his point of view thusly:

There can be little doubt that the maintenance and regulation of activity of the anterior pituitary gland is dependent on the gland being supplied with blood via the hypophyseal portal vessels. The information as to the details of the mechanism involved is scanty but it seems likely that nerve fibers in the hypothalamus liberate some humoral substance into the primary plexus of the vessels, and that this substance is carried by the vessels to affect anterior pituitary activity.

Zuckerman (175), on the other hand, adopts the position that

... a direct vascular connection between the base of the brain and the pars distalis

is neither sufficient, other things being equal, nor necessary for positive responses of the pars distalis.

Glydon (65) has studied the embryology of the portal vessels in the fetal rat. He finds they appear several days before birth but the rich capillary network in the median eminence does not begin to develop until the fifth postnatal day. Glydon cites studies by others which demonstrate that the fetal pituitary-adrenal system is functional and concludes that the fetal pituitary of the rat can function in the absence of the distinctive vascular arrangement of the adult median eminence. Consideration of the data of Jailer (88) and of Rinfret & Hane (139) suggests that although qualitatively the same, adrenal ascorbic acid depletion is quantitatively less in the neonate than in the adult rat. These observations together with those of Glydon would support the thesis that the portal venous system is necessary for adequate function of the pituitary adrenal system.

Daniel & Prichard (33, 34, 35) find that in man, sheep, and rat the epithelial cells of the anterior lobe are supplied exclusively with portal venous blood. Interruption of these vessels either by coagulation or stalk section results in extensive necrosis of the adenohypophysis. Greep & Barnett (12, 68) found that interruption of the portal vessels in the rat resulted in extreme cellular necrosis and degeneration in the adenohypophysis together with functional changes in the target endocrine glands. Only a narrow peripheral rim of pituitary tissue remained intact 24 hr. after stalk section. In their studies there seems to have been some proliferation of the remaining tissue, but fifteen days after vessel interruption, the gland showed marked diminution in size and contained a dense connective tissue scar in the central area. No quantitative estimate of loss of glandular tissue was made. They point out that ischemia itself may be sufficient to account for changes in pituitary function following stalk section. Campbell & Harris (27) demonstrated a 17 to 32 per cent reduction in the volume of the pars distalis of the rabbit 48 to 160 days after stalk section. Apparently a lesser amount of infarction occurs in the adenohypophysis of the rabbit than in man, sheep or rat following stalk section. This may possibly be attributed to partial regeneration of the glandular elements after extensive necrosis or to the fact that the rabbit adenohypophysis receives some of its blood supply directly from arteries (75).

Smith in 1932 (151) showed that removal of 70 per cent of the rat adenohypophysis did not consistently result in atrophy of the thyroid, adrenals, or gonads. Recently, Ganong & Hume (59) reported on the effects of graded hypophysectomy in the dog. They observed that losses of pituitary tissue approaching 90 per cent may not result in adrenal atrophy. Precise estimates of residual pituitary tissue are wanting in this study since the control seems to be limited to one dog. The studies of Smith and of Ganong & Hume demonstrate that the adenohypophysis possesses a great deal of functional reserve and that endocrine changes following stalk section may not be a consequence of diminished pituitary volume per se.

Fortier, Harris & McDonald (53) have conducted a series of experiments designed to evaluate the consequence of stalk section on the discharge of ACTH in response to a variety of stimuli. Stalk section was performed on adult female rabbits via a fronto-temporal approach which avoids disruption of the direct arterial supply of the pituitary and of the venous drainage of the gland. In some of the animals a waxed-paper plate was introduced between the hypothalamus and the pituitary in order to prevent the reestablishment of the portal venous system. Despite the presence of a plate, histologic examination at the conclusion of the experiment revealed a fine capillary communication between the primary plexus of the median eminence and the pars distalis in all but one animal. These stalk-sectioned animals with plates showed no lymphopenic response to restraint or exposure to cold, but did show a significant but reduced response to epinephrine administration and to laparotomy under ether anesthesia. Rabbits with stalk section, but without the intervening plate, similarly showed no lymphopenic response to restraint. The latter group was not tested for their response to cold, epinephrine, or laparotomy. However, they were retested for their response to restraint at subsequent times. The capacity of these animals to respond seemed to increase with time but even after two months did not reach the level of intact controls. In the case of the stalk-sectioned animals with plate no time studies were performed to show that a similar return of function did not occur. In fact, there is nothing to assure the reader that the difference in response between restraint and cold on the one hand and epinephrine and laparotomy on the other were not due to the time sequence in which these studies were performed. It was further found that stalk-sectioned rabbits with plates responded to the stimulus of unilateral adrenalectomy with adrenal ascorbic acid depletions equal to those observed in normal controls. Thus, Zuckerman's contention that a direct vascular connection between the base of the brain and the pars distalis is not necessary for positive responses of the pars distalis is borne out. However, Fortier *et al.* conclude that the portal venous connection between the hypothalamus and the pituitary is necessary for ACTH release in response to "neurotropic" stimuli (restraint, cold) but not necessary for the response to "metabolic" stimuli (epinephrine, laparotomy). We find no logical basis for such a classification of stimuli. Restraint or forced immobilization is usually accompanied by vigorous muscular activity which is in turn accompanied by epinephrine discharge and the release of products of muscular activity. Restraint is just as logically classified "metabolic" as "neurotropic." Cold is usually considered to be a stimulus which induces numerous metabolic alterations in animals and it is of interest that Fortier (51) in an earlier study so classified this stimulus. The actual basis for classification appears to be the response of the "isolated pituitary." This is apparent from the authors' statement that

These findings (lymphopenia or adrenal ascorbic acid depletion) support the view that environmental stimuli may be divided into two types: those that affect ACTH secretion solely by an action through the central nervous system, and those that act also by affecting the composition of the blood in the systemic circulation.

By the same type of reasoning it is possible to classify stimuli by intensity rather than modality. The act of isolating the pituitary by stalk section or transplantation may damage the gland to the extent that it is capable of responding only to intense stimuli. One could then conclude that restraint and exposure to cold are less intense than epinephrine injection or laparotomy since stalk-sectioned animals only respond to the latter two stimuli.

Brown-Grant, Harris & Reichlin (26) have used the same stalk-sectioned rabbits described in connection with ACTH release to evaluate the role of the portal venous system in the regulation of TSH discharge. Thyroid I^{131} uptake was normal in the simple stalk-sectioned rabbits, but less than normal in the stalk-sectioned rabbits with plate. On the other hand, release of I^{131} from the thyroid was impaired to the same degree in both groups. The discrepancy between the two tests of thyroid function remains unexplained. An interesting feature of these studies is the fact that the regeneration of the portal system after simple stalk section was not associated with normal thyroid function as evaluated by I^{131} release. Furthermore, disruption of portal venous connections by a plate did not impair the inhibitory influence of laparotomy on I^{131} release. The data suggest that the portal system is not an essential link for inhibition of release of TSH. The stimulus of restraint induced a temporary decrease in the release of thyroid I^{131} in 21 of 23 experiments on 19 normal rabbits but failed to induce a decreased rate of release in 22 of 33 animals with stalk section and plate. The differences between the responses to laparotomy and restraint are judged by the authors to be in accord with the concept that the former is a "metabolic" and the latter a "neurogenic" stimulus. This reasoning applied to TSH release is subject to the same criticisms as when applied to ACTH release.

The stalk-sectioned rabbits used in the preceding two studies showed severe adrenal atrophy whether they had undergone simple stalk section or stalk section with insertion of a waxed-paper plate. The degree of adrenal atrophy was equal in both groups. Since the authors state that

The simple stalk sectioned rabbits, as also the normal rabbits, showed highly developed vascular connections between the median eminence and the pars distalis, which appeared to form a major part of the blood supply to the gland,

it is obvious that the intactness of the portal system does not determine adrenal weight maintenance or ability of the thyroid to release I^{131} . Thus, it would seem that Zuckerman's (175) second contention that, other things being equal, a direct vascular supply between the adenohypophysis is not sufficient for positive responses of the pars distalis, is also confirmed.

In marked contrast to the adrenal atrophy seen in the stalk-sectioned rabbits by the British workers is the observation by McCann (103) that lesions in the hypothalamus of the rat which prevent adrenal ascorbic acid depletion to many stimuli are accompanied by normal or enlarged adrenal cortices. Harris (77) refers to adrenal atrophy as indicating "... that the basic resting secretion of ACTH was much reduced by the operative proce-

dure of stalk section." By this criterion then, McCann's lesioned rats had normal or elevated resting secretions of ACTH, and yet did not respond to the stimulus of unilateral adrenalectomy, histamine, or epinephrine injection with depletions of adrenal ascorbic acid. We suggest that the adrenal atrophy of the British workers may be secondary to other endocrine changes induced by stalk section.

Brown-Grant *et al.* (26) state that, "... there was no obvious relationship between these indices for thyroid activity and adrenal weight in the stalk sectioned rabbits." The significance of this statement is difficult to appreciate because examination of the data indicates that stalk-sectioned rabbits (with or without plates) exhibited both a marked degree of adrenal atrophy and a marked reduction in the rate of I^{131} release from the thyroid. The possibility must be considered that adrenal atrophy which followed stalk section was not a primary defect in adenohypophyseal function, but an expected response of the pituitary or adrenal to a state of hypothyroidism. It is tempting to speculate that in lesions involving disruption of the portal venous system a selective destruction of the glandular elements occurs. Purves & Griesbach (128) picture the thyrotrophs as occupying a central location within the gland. It is the central area which Daniels & Pritchard (33) have shown to be characteristically involved in ischemic necrosis of the rat adenohypophysis following stalk section. Leblond & Hoff (95) have shown that thyroidectomized animals have definite adrenal atrophy. Gabrilove & Soffer (57) found that hypothyroid rats with marked adrenal atrophy could respond to large doses of epinephrine or ACTH with a significant adrenal ascorbic acid depletion. Thus, the data of the British workers could be explained on the basis of hypothyroidism resulting from selective destruction of the pituitary subsequent to stalk section.

The preliminary studies of Nikitovitch & Everett (119) confirm the earlier work of Harris & Jacobsohn (78) which demonstrated that pituitary grafts function best when implanted adjacent to the median eminence in hypophysectomized rats. Nikitovitch & Everett grafted pituitary fragments to the kidneys of hypophysectomized female rats. Anestrus developed. Two weeks later some of the grafts were retransplanted into the sella turcica. Five of the animals had a resumption of estrus cycles within 40 to 73 days. Animals whose pituitaries were not returned to the sella turcica remained in anestrus. If confirmed, these studies will lend strong support to Harris' thesis (76).

Systemic circulation.—This will be dealt with in a subsequent section.

CHEMICAL BLOCK OF ACTH RELEASE

STEROID BLOCK

Extreme changes in plasma levels of corticosteroids have been shown to influence ACTH release. At the one extreme, adrenalectomy is followed by increased plasma levels of ACTH; at the other, administration of large quantities of corticosteroids inhibits ACTH release. These observations have

led to the theory that ACTH release may be regulated fully or in part by fluctuating corticosteroid titers in the peripheral blood. In order to validate this theory it must be demonstrated that stimuli capable of eliciting ACTH release do indeed result in increased peripheral utilization⁶ of biologically active corticosteroids and that small changes in peripheral blood titers of corticosteroids are capable of influencing ACTH release. Recent studies have done little more than retest the reciprocal relationships between extreme changes in plasma corticosteroid levels and ACTH release.

Corticosteroids.—One week after unilateral adrenalectomy in the dog, Ganong & Hume (60) found hypertrophy and an increased rate of 17-hydroxycorticosteroid secretion from the remaining adrenal. Two hours after unilateral adrenalectomy these changes were not observed in the remaining gland. The absence of an increased secretion rate of 17-hydroxycorticosteroids after the shorter time interval does not necessarily mean that the pituitary is unable to respond rapidly to decreased production of corticosteroids. In this study it is apparent that the remaining adrenal was already functioning maximally as a consequence of the cannulation process and further release of ACTH could not result in increased 17-hydroxycorticosteroid output.

Barrett & Hodges (9, 10) were unable to detect elevated resting blood levels of ACTH in rats up to 12 days after adrenalectomy. They could also find no difference in the levels of blood ACTH attained by adrenalectomized and by intact rats following the stimulus of anesthesia and laparotomy. The discrepancy between these findings and previous reports (160) of elevated blood levels of ACTH following adrenalectomy may be due to an insufficient period of time elapsing between adrenalectomy and testing. The reason for the necessity of allowing from two to three weeks to elapse before detectable levels of ACTH appear in the resting adrenalectomized rat is obscure. Perhaps pituitary synthesis is unable to keep up with increased rates of release of ACTH in the early period following adrenalectomy. A preliminary report by Fortier (52) indicates that the ACTH content of the pituitary declines for the first 20 hr. following adrenalectomy. ACTH content returns to control levels by the fourth postoperative day and by the thirty-second day the pituitary ACTH content is five times control level. ACTH assays were done by the *in vitro* technic.

By infusing dogs with cortisol (hydrocortisone), Richards & Pruitt (137) were able to prevent the increase in adrenal vein 17-hydroxycorticosteroids which normally follows adrenal vein cannulation or exposure to an atmosphere of 20 per cent CO₂. It appears that the infusions were at a rate which is about ten times the estimated 17-hydroxycorticosteroid output of both maximally stimulated canine adrenals and resulted in 17-hydroxycorticosteroid plasma levels which were three to four times higher than those seen under maximal stimulation in the normal dog.

The use of massive doses of adrenal steroids for the inhibition of ACTH

⁶ Utilization is meant to include the sum total of all processes which result in a reduction of biologically active corticosteroids.

release in test animals continues. The studies of Barrett & Hodges (9, 10) make use of rats pretreated with desoxycorticosterone for the assay of ACTH. Mialhe-Voloss (109) finds that pretreatment of rats with large quantities of cortisol prevents the decrease in adrenal ascorbic acid which otherwise follows the application of an auditory ("neurogenic") stimulus or the administration of histamine ("systemic stimulus"). Mialhe-Voloss & Stutinsky (110) report that such pretreatment also blocked adrenal ascorbic acid changes following the intraperitoneal administration of 0.5 unit of Pitressin, but not following the administration of larger quantities of Pitressin or the combination of unilateral adrenalectomy and histamine. The relative rather than the absolute ability of the corticosteroids to block ACTH release is implied in the authors' statement that "... unilateral adrenalectomy followed by an injection of histamine constitutes a stress of such magnitude that we are at the limit of blockage." (Reviewers' translation.)

Androgens.—Brown & Migeon (25) observed that infusions of massive quantities of testosterone into normal male subjects resulted in a profound fall in plasma 17-hydroxycorticosteroids during the half hour following the infusion. Progesterone had little or no effect. The response of plasma 17-hydroxycorticosteroids to ACTH was normal. Zizine (174) has confirmed this property of testosterone. He pretreated rats with large amounts of testosterone for six days. On the seventh day the rats failed to respond to unilateral adrenalectomy with a depletion of adrenal ascorbic acid although they retained their ability to respond to an unspecified dose of ACTH. Thus it seems that testosterone is capable of inhibiting ACTH release, but the site of action and the possible physiologic significance of the inhibition is obscure.

DRUG BLOCK

Chemical agents which may induce inertia of the pituitary-adrenal system can be classified into three groups: (a) Agents acting directly on adrenal cortex. Certain substituted estrogens (106, 167) and amphenone (81, 164) have a direct inhibitory effect on the adrenal cortex and inhibit corticosteroid secretion in response to ACTH. Consequently these agents should be capable of inhibiting corticosteroid secretion in response to any stimulus and would be of little value in the study of neural factors initiating ACTH release. (b) Agents specifically blocking particular drugs. ACTH release following administration of epinephrine, methacholine (70), and histamine (163) may be inhibited by the prior administration of their respective antagonists: adrenergic blocking agents, cholinergic blocking agents or antihistaminics. These blocking agents would be expected to inhibit only ACTH release mediated or stimulated by the drugs which they antagonize. (c) Agents presumably acting on the central nervous system. Morphine, reserpine, and chlorpromazine are capable of blocking ACTH release to a wide variety of stimuli. This observation and the presumption that their site of action is on the hypothalamus suggests that they may be of value in studying nervous control of ACTH release.

Morphine.—Briggs & Munson (20) found that pretreatment of rats with

pentobarbital and large but sub-lethal doses of morphine results in inhibition of adrenal ascorbic acid depletion usually observed following the administration of histamine, epinephrine, Pitressin, as well as after laparotomy and unilateral adrenalectomy. In their studies it is clearly shown that effectiveness of the drug block is a function of stimulus intensity and not absolute. Small doses of histamine were ineffective in inducing adrenal ascorbic depletion, but significant depletions followed massive doses of histamine in the "blocked" animal.

Ohler & Sevy (120) have confirmed the efficacy of the pentobarbital-morphine combination in blocking adrenal ascorbic depletion in response to sham and unilateral adrenalectomy and to Pitressin and epinephrine administration.

Tranquilizers.—Wells, Briggs & Munson (171) found that in the rat, administration of reserpine for five days results in marked inhibition of ACTH release which follows administration of histamine or ether. The effective dose of reserpine caused notable weight loss in the animals during the five day treatment period. Wells *et al.* claim that the action of reserpine is not on the adrenal cortex since four milliunits of ACTH given intravenously resulted in adrenal ascorbic acid depletion. In the one experiment shown the reserpine treated animals appeared less responsive to the single dose of ACTH which was given. The reviewers feel that a question as important as this deserves a multi-dose sensitivity test.

Olling & de Wied (121) found that chlorpromazine following sodium pentobarbital prevents, at least partly, the release of corticotropin brought about by histamine, epinephrine, norepinephrine, or unilateral adrenalectomy. This inhibition does not seem to be due to depression of adrenal cortical function. The authors discuss the possible site of action of chlorpromazine and come to no definite conclusion. Chlorpromazine preceded by sodium pentobarbital was found by Sevy, Ohler & Weiner (147) to inhibit adrenal ascorbic acid depletion following sham and unilateral adrenalectomy and epinephrine injection. Chlorpromazine did not inhibit ACTH release following the intravenous administration of Pitressin and it is concluded that chlorpromazine is ineffective against this agent. In the same study it was shown that the blocking effect of chlorpromazine is dependent on the dose used. This finding may explain the ineffectiveness of chlorpromazine in blocking the excitatory effect of Pitressin. The authors anticipate this objection and attempt to discount it. Another series of experiments with larger doses of chlorpromazine could have settled this point.

The blocking effects of chlorpromazine have been studied in man by Christy and co-workers (32). They found that the administration of chlorpromazine to schizophrenic patients two hours prior to insulin coma therapy prevented the usual rise in plasma 17-hydroxycorticosteroids. Chlorpromazine was thought not to act on the adrenal cortex since chronic administration did not depress the ability of large doses of ACTH to elevate the peripheral blood steroid levels.

In addition to their ability to inhibit ACTH release, reserpine and chlorpromazine have also been shown to elicit ACTH release. Thus, these agents, like morphine (20), have an excitatory as well as a depressant effect on the ACTH release mechanism.

Wells, Briggs & Munson (171) point out that the acute administration of reserpine results in ACTH release in the rat. Harwood & Mason (79) observed a rise in the concentration of 17-hydroxycorticosteroids in the unanesthetized monkey after the administration of reserpine. Rosenthal & Mason (141) reported a marked increase in urinary output of 17-hydroxycorticosteroids following the administration of reserpine to monkeys.

Olling & de Wied (121) found that chlorpromazine given intravenously to trained unanesthetized rats causes significant adrenal ascorbic acid depletion which is almost completely blocked by pretreatment with sodium pentobarbital. Sevy, Ohler & Weiner (147) have injected chlorpromazine into untrained, unanesthetized rats and noted adrenal ascorbic acid depletions no greater than those after saline administration. Since the animals were not previously adapted to handling, the saline control rats showed marked adrenal ascorbic acid depletions which may have obscured a specific effect of chlorpromazine. Egdaahl & Richards (41) found that chlorpromazine injected intravenously resulted in an acute rise in adrenal vein 17-hydroxycorticosteroids in the unanesthetized dog. This action was abolished by hypophysectomy but not by the infusion of cortisol. The authors suggest that the drug may act either on the hypothalamus or directly on the pituitary.

We agree with Briggs & Munson (20) that "There is no evidence that the hypothalamus is the site of action of morphine," and would extend this statement to include the tranquilizing drugs as well as the corticosteroids. Furthermore, in the case of all these agents a direct effect on the pituitary cannot be ruled out. These two considerations together with the relative rather than absolute blocking ability of these agents seem to limit the application of the drug blocking technique to the problem of ACTH release.

INITIATION OF CORTICOTROPIN RELEASE AND THE CORTICOTROPIN RELEASING FACTOR (CRF)

The discussion has been limited to the neural pathways which convey stimuli to the hypothalamus and to the connecting links which transmit hypothalamic influence to the adenohypophysis. We now propose to discuss possible ways by which ACTH release can be regulated. A classification is presented which includes Excitatory Factors, Inhibitory Factors and a Trophic Factor (trophic used in the sense of nurturing influence). The designation, Corticotropin Releasing Factor (CRF), a term suggested by Saffran, Schally and Benfey (144), we propose to limit to a substance which has a relatively specific action on the adenohypophysis to release ACTH because of its unique mode of transport, its elaboration in the adenohypophysis or its peculiar chemical structure.

EXCITATORY FACTORS

CRF via portal venous system.—CRF is elaborated in the hypothalamus, possibly in or near the median eminence, and carried by way of the portal venous system to the adenohipophysis. The specificity of the mechanism, i.e., its capacity to excite the adenohipophysis to release ACTH,⁶ resides in the mode of transmission of CRF via the portal venous system. CRF need not have a unique chemical structure. The substance may be a familiar, well characterized neurohumor such as acetylcholine or epinephrine, or a polypeptide such as antidiuretic hormone. The concept of a neurohumor passing from the median eminence to the adenohipophysis via the portal venous system now dominates thinking in the field of adenohipophyseal regulation.

CRF at nerve endings.—CRF is elaborated at nerve endings in the adenohipophysis. Impulses pass by way of a neural path connecting a hypothalamic center with the adenohipophysis. The transmission by a neural path endows the mechanism with the unique property of stimulating the adenohipophysis and the substance elaborated may be a neurohumor already characterized as playing a role of transmitter in other areas of the body. The concept has not gained wide acceptance because of lack of convincing evidence for innervation of the adenohipophysis.

CRF via systemic circulation.—CRF is transmitted via the systemic circulation from one or more organs (brain, liver, etc.). According to this thesis, stimuli or traumata, acting at the periphery, result in elaboration of a substance which because of its unique chemical structure acts on adenohipophyseal cells to induce release of ACTH. The capacity of adenohipophyseal transplants in the anterior chamber of the eye or in the spleen to release ACTH in response to certain stimuli may be adequately explained by such a mechanism.

Metabolites or toxins.—A variety of metabolites or toxins carried from the periphery via the systemic circulation may act directly on adenohipophyseal cells to bring about release of ACTH. The experimenter is inclined to revolt at the thought of such a crude mechanism acting to induce release of ACTH, yet the direct action of a variety of agents on adenohipophyseal cells is consistent with the nonspecific character of the response of the pituitary. If such a mechanism exists in addition to a more elegant mode of inducing ACTH release, it is readily understandable why we are having difficulties sorting out specific as against nonspecific factors acting on the adenohipophysis.

INHIBITORY FACTORS

In common with a number of processes in the organism, the regulation of ACTH release has an inhibitory as well as an excitatory component.

⁶ The reviewers have left out of consideration the regulation of release of tropic hormones other than ACTH. The complexity of the problem of release of one tropin to the exclusion of another precludes consideration in this review.

Corticosteroid inhibition.—Corticosteroid inhibition of ACTH release is established beyond doubt. However, we still lack a clear definition of the role of this inhibitory factor on the minute-to-minute regulation of ACTH release and a knowledge of whether its site of action is the adenohypophysis or the hypothalamus. Actually, the possibility has not been ruled out that a metabolic consequence of the action of the corticosteroids or a degradation product of the corticosteroids rather than the corticosteroids themselves acts to inhibit ACTH release.

Hypothalamic inhibition.—Hypothalamic inhibition of ACTH release, apart from corticosteroid activity, is a distinct possibility. There may be a hypothalamic inhibitory as well as an excitatory center.

TROPHIC FACTORS

The hypothalamus may have a trophic (in the sense of nurturing) influence which maintains the functional integrity of the cells of the adenohypophysis and renders them responsive to an extra-hypothalamic factor which excites release of ACTH. The influence would be analogous to that of estrogen on the responsiveness of the smooth muscle of the uterus to the acute action of oxytocin. The apparently special property of the sella in maintaining functional pituitary transplants may be accounted for by the action of a trophic factor from the hypothalamus. The findings of Nikitovich & Everett (119) and of Harris & Jacobsohn (78) that transplants in the sella have a higher degree of functional capacity than those at other sites in the body are compatible with such a thesis.

HEMODYNAMIC AND PHYSICOCHEMICAL FACTORS

The portal venous system may exert a regulatory influence on ACTH release, not by carrying a neurohumor, but by adjusting blood flow through the adenohypophysis. Vasoconstrictors or vasodilators released about the loops of the portal system in the median eminence could conceivably regulate ACTH release by adjusting blood flow to the gland. Other possibilities, including PCO_2 , PO_2 , and pH of the blood carried in the portal system, cannot be excluded.

COMBINATION OF FACTORS

Any combination of the above mentioned factors or mechanisms may operate to regulate release of ACTH. An indication of the status of investigation in the field is the fact that we cannot at present definitely rule out any one of these proposed mechanisms for regulation of ACTH release.

THE ASSAY OF CRF

The various attempts at the isolation or identification of CRF, will be evaluated according to the test object employed for its assay. The significance of experimental data is in large measure determined by the qualifications of the test animal. According to McCann (102),

... the ideal assay animal to test for possible neurohumoral activators of ACTH release would be one in which the ubiquitous pituitary-adrenal response to stress was completely blocked while leaving unimpaired the sensitivity of the pituitary and adrenal to the neurohumor and ACTH, respectively.

No experimental study has met these requirements for an ideal assay with the possible exception of those of McCann (102).

In vitro pituitary.—Saffran & Schally (143) have obviated the complexities of *in vivo* assay. They consider the capacity of a substance to increase the quantity of ACTH in the medium of an incubated anterior lobe of pituitary to be the most direct measure of its *in vivo* function as a CRF. Hypothalamic tissue plus norepinephrine, but neither one alone, increased the quantity of ACTH in the medium of incubated pituitary as compared to the medium of control pituitary with no additions. The combinations, cerebral cortex plus norepinephrine and posterior pituitary tissue plus norepinephrine, were also active. CRF has been separated from vasopressin by paper chromatography (144). Neither histamine nor purified vasopressin is active in the *in vitro* system (146).

Guillemin and coworkers (71) report the presence of CRF in hypothalamus, posterior pituitary, substance P, and cerebral cortex when assayed by the *in vitro* pituitary test of Saffran & Schally (143). On the basis of chromatographic mobility, Guillemin *et al.* claim that the CRF activity of these various source materials resides in a common fraction (fraction D). "The purified material appears to be a small peptide, different from vasopressin, oxytocin, ACTH, histamine, acetylcholine, adrenalin, noradrenalin, and 5-hydroxytryptamine by its pharmacological and chromatographic characteristics."

Swingle *et al.* (158, 159) have demonstrated that Pitressin, a hydrolysate of Pitressin, substance P and a hydrolysate of substance P will increase the ACTH content of the medium of incubated pituitaries. Since publication the authors have expressed doubts as to the validity of the test (personal communication); materials other than those mentioned above give positive reactions in the *in vitro* test. Barrett & Sayers (11), on the basis of *in vitro* studies on ACTH degradation, suggest that the so-called CRFs are not accelerating release of ACTH *in vitro* but inhibiting its degradation, possibly by competition for a proteolytic enzyme. In the words of Guillemin and his coworkers (71):

A critical appraisal of the physiological meaning of all these results obtained *in vitro* appears to be necessary; their true significance, if any, will have to be assessed in some dynamic fashion lest they remain of purely esthetic value.

Intact animal.—The intact animal is of no value for the screening of excitatory neurohumors since practically any stimulus will induce adrenal ascorbic acid depletion in this test animal. A possible exception to this generalization is a test in the intact animal which judges ACTH release on the basis of elevation of blood ACTH. Even the most intense stimuli pro-

duce no more than a just detectable quantity of ACTH in the blood of the intact rat. A substance which induces an appreciable increase would be of some interest. In this connection Brodish & Long (24) report that the blood of hypophysectomized rats contains a factor which will significantly increase the level of circulating ACTH in the intact rat as judged by bioassay of the blood. The factor is not found in the blood of decapitated, hypophysectomized rats nor is this factor found in the blood of intact rats.

Steroid "blocked" animal.—If the action of the corticosteroids is at the level of the hypothalamus to inhibit release of CRF then there is real justification for use of such a test object. On the other hand, if the steroids block the excitatory action of CRF on adenohypophyseal cells, the conditions defeat the purpose of the assay. One gets the distinct impression that the effectiveness of the block is related more to the intensity of the stimulus than the type or quality of the stimulus, i.e., any stimulus, be it drug, toxin, or trauma, will break through the block if it is of sufficient intensity.

Porter & Jones (125) have employed the hydrocortisone "blocked" rat to assay plasma of blood collected from the cut end of the pituitary stalk. Adrenal ascorbic acid depletion induced by two 2 ml. doses of plasma from sella blood was of a very low order of magnitude, but statistically significant. Plasma from carotid blood was without effect on adrenal ascorbic acid. The active factor in plasma from sella blood is not dialyzable; it corresponds to Cohn's fraction II plus III (126). Commercial Pitressin in a dose of 1.2 to 2.4 units induced depletion of adrenal ascorbic acid in the hydrocortisone "blocked" rat. However, unlike Pitressin, the active factor in sella blood plasma did not induce blanching. Two objections may be raised in connection with these studies. First, the specificity of the steroid "blocked" rat for the assay of CRF had not been established. Second, in these particular experiments, plasma from blood oozing at a site of tissue damage is a more appropriate control than plasma from carotid blood.

Stutinsky (155) claims the presence of CRF in extracts of hypothalamus as judged by adrenal ascorbic acid depletion in hydrocortisone treated rats. The author concludes that the active principle is not antidiuretic hormone.

Drug "blocked" animal.—The use of an agent such as morphine to assay for CRF is predicated on the hope that the drug acts on the hypothalamus to inhibit release of the neurohumor in response to nonspecific stimuli. Neuropharmacological studies of morphine, barbiturates, and tranquilizers have not revealed the site or mode of action of these agents. In the absence of such knowledge the drug "blocked" animal has no sound basis as an assay object for CRF. As in the case of corticosteroid "block" one gets the impression that the effectiveness of the block is determined more by the intensity than the type of stimulus. The adrenal ascorbic acid depleting activity of 0.07 unit of Pitressin is abolished by pretreatment of the rat with morphine (20). However, the conclusion derived from such an experiment to the effect that Pitressin does not contain CRF has been challenged since a greater dose, 0.5 unit of Pitressin, will break through the morphine

block (102). The capacity of large doses of histamine as well as Pitressin to break through a morphine block may be accounted for by a direct action of these vasoactive agents on the adenohypophysis.

"Isolated" pituitary.—The pituitary isolated from the hypothalamus by transplantation to the anterior chamber of the eye (30), by stalk section (29) or by introduction of a plate between the hypothalamus and the pituitary (53) responds with ACTH release to various stimuli and agents. Therefore, it is not a suitable test object for CRF. However, it would be of interest to determine whether ACTH can be released from a pituitary transplant in the anterior chamber in an animal with an "effective" lesion in the median eminence. If such a lesion blocked release of ACTH from the transplant, the concept of CRF elaboration by the hypothalamus would be strengthened considerably.

Hypothalamic lesion animal.—An animal with a lesion in the center which elaborates CRF obviously represents the test animal of choice for the assay of the substance provided the only deficiency or impairment of consequence is the loss of CRF. McCann & Brobeck reported in 1954 (103) that a lesion in the supraopticohypophyseal tract induces diabetes insipidus and inertia of the pituitary-adrenal system in rats. The animals failed to exhibit adrenal ascorbic acid depletion in response to unilateral adrenalectomy alone or in combination with 3 mg. of histamine phosphate, 5 μ g. of epinephrine or five units of Pitocin. Adrenal ascorbic acid depletion was induced in these lesioned rats by five units of Pitressin and McCann suggested that the antidiuretic hormone is CRF. In a recent communication, McCann (102) presents confirmation and extension of these experiments and provides experimental data relevant to the sensitivity of the adrenals of the lesioned rat to exogenous ACTH. Reduction in sensitivity of the adrenal to ACTH must be ruled out before definitive conclusions are made to the effect that an impairment in the release of ACTH is characteristic of an animal with diabetes insipidus. Five milli-units of ACTH administered intravenously induced on the average less adrenal ascorbic acid depletion in rats with diabetes insipidus than in hypophysectomized rats or in rats "blocked" with the combination sodium pentobarbital plus morphine. However, the response in the rats with diabetes insipidus was so variable that the mean, although low, was not significantly different from that of either of the other two groups of animals. These sensitivity studies, carried out three weeks after placement of the lesion, should be extended in order to settle a most important question.

Correction of the diabetes insipidus with daily injections of Pitressin in oil did not correct the lack of response to unilateral adrenalectomy. McCann concludes that it is not the diabetes insipidus per se but the lack of ADH in sufficient concentration in the portal venous system that is responsible for the inertia of the pituitary.

McCann (102) has recently introduced the "acute" diabetes insipidus rat as a test object for CRF. Forty-eight hours after placement of the lesion, unilateral adrenalectomy plus histamine, epinephrine, or substance P failed

to induce a response. Pitressin and Protopituitrin were active. McCann claims the adrenal of the "acute" diabetes insipidus rat responds with normal sensitivity to ACTH. The reviewers consider the evidence of normal adrenal sensitivity to ACTH suggestive but not conclusive and propose a multidose technic for the resolution of this crucial question.

Pitressin was resolved into various chromatographic fractions by McCann. Assay in the "acute" diabetes insipidus rat demonstrated that CRF activity resides in the fraction high in vasopressin activity. This is contrary to the findings of Guillemin *et al.* (71) who claim that CRF is a contaminant of vasopressin in commercial Pitressin. Guillemin's highly potent CRF, fraction D, was inactive in the "acute" diabetes insipidus rat of McCann.

McCann (102) has marshalled the following evidence in favor of the concept that ADH is the neurohumor (CRF) which induces release of ACTH:

"(1) Release of ADH occurs during the stressful situations associated with release of ACTH (62, 111, 166) and this release of ADH probably occurs quickly enough to account for the rapidity of release of ACTH (46, 111, 160). 2) There is a correlation between the reduction in ADH release, as judged by severity of diabetes insipidus and the reduction in ACTH release in rats with hypothalamic lesions (103, 104). 3) Pitressin, a commercial extract containing the pressor-antidiuretic activity of the posterior lobe, is effective in inducing ACTH release in rats with hypothalamic lesions which block the response to nonspecific stimuli (103). The dose of Pitressin required to elicit ACTH discharge in rats with lesions is very large, suggesting that the amount of endogenously liberated ADH would be effective only if the ADH reached the pituitary in high concentrations via the hypophyseal portal veins after its secretion in the median eminence of the tuber cinereum."

The evidence is impressive, particularly since McCann's test assay has fewer objectionable features than any other now in use. However, the reviewers are not willing to accept ADH as the corticotropin releasing factor without reservation for the following reasons: (a) Pitressin fails to induce elevation of blood ACTH in the adrenalectomized rat with a high pontine section, whereas epinephrine and an extract of hypothalamus are active in this test animal (145). (b) Administration of large doses of water (which inhibit ADH release) induce ACTH discharge (113). (c) Epinephrine inhibits ADH release (40) but stimulates ACTH discharge. (d) Discharge of ACTH from a pituitary transplant in response to histamine (30) is difficult to explain in terms of McCann's thesis. According to McCann, ADH reaches effective concentrations for ACTH discharge in the portal venous system but not in the systemic circulation when the animal is subjected to nonspecific stimuli. A transplant in the anterior chamber, far removed from the median eminence, would not be expected to receive sufficient ADH to induce ACTH discharge. (e) According to McCann (102), Pitressin does not induce adrenal ascorbic acid depletion in the hypophysectomized rat. On the contrary, Sayers (unpublished observations) claims Pitressin has 0.1 milliunit of ACTH activity per unit of vasopressin activity, and Nelson & Hume (117)

have reported that Pitressin increases the secretory activity of the adrenal cortex of the hypophysectomized dog (adrenal vein 17-hydroxycorticosteroids as measured by the Nelson-Samuels technic). That the ascorbic acid depletion induced by Pitressin is due to ACTH contamination has not been unequivocally ruled out.

The experimental data at hand are inconclusive in regard to the nature of CRF. We must await further delineation of the limitations and potentialities of present methods or the development of new assay methods for CRF before definitive statements can be made as to the identity or even the existence of such a neurohumor.

THE NATURE AND THE REGULATION OF THE SECRETION OF THE ADRENAL CORTX

In the few years that have elapsed since the introduction of micro methods for the isolation and quantitation of the corticosteroids our knowledge of the secretion of the adrenal cortex has passed from the status of speculation to an impressive body of solid facts. Cortisol, corticosterone, and aldosterone are now established as the adrenal secretory products of major biological importance in dog (47) and man (84, 140, 148). We may conclude from quantitative estimates of the rate of secretion of cortisol that this steroid accounts in large measure for the action of the adrenocortical secretion on carbohydrate and protein metabolism. On the other hand, aldosterone is in large part responsible for the action of the adrenal cortex on electrolyte metabolism. However, corticosterone cannot be discounted. In the dog and in man, this steroid, of moderate, but dual activity, is secreted at a rate sufficient to make a small but significant contribution to both of the major metabolic activities of the adrenal cortex.

The role of the pituitary in the regulation of the rate of secretion of these steroids has been defined (47, 97) and a controversial issue, the question of division of function between the zones of the adrenal cortex, has been in part resolved. The concept of Deane, Shaw & Greep (37), based on histochemical technics, that a sodium-retaining factor is secreted by the glomerular zone of the adrenal cortex, is supported by two groups of workers (7, 63) who have demonstrated by *in vitro* methods that the outer zone of the adrenal cortex, composed mainly of glomerulosa, synthesizes aldosterone at a significantly higher rate than the rest of the cortex.

17-Ketosteroids have been isolated from adrenal vein blood of the dog (80) and of man (140). However, a statement as to the physiological significance of these substances must await results of experiments designed to (a) quantitatively estimate their rate of secretion, (b) define mechanisms which regulate their secretion, and (c) assess their androgenic activity.

SECRETION RATE UNDER BASAL CONDITIONS

A quantitative appraisal of the fluctuations in the secretory activity of the adrenal cortex depends upon the availability of estimates of the rate of

secretion of the gland in the basal as well as the stimulated state. This poses the technically difficult problem of securing a sample of adrenal vein blood from an unanesthetized, unexcited animal. Hume & Nelson (86) have devised a technic for diverting flow in the adrenal vein to the exterior for short periods. A "choker" occludes the adrenal vein between the adrenal and the vena cava and blood flow is directed to the exterior via a cannula in the lumbo-adrenal vein. Egdahl & Richards (41, 42) have employed this technic to collect small samples of adrenal vein blood from unanesthetized dogs 24 hr. after recovery from the operative manipulations attendant on cannulation. In one group of 16 dogs the average output of 17-hydroxycorticosteroids (in large measure cortisol) was $6.4 \mu\text{g./kg./hr.}$,⁷ and in another group of six, the average output was $8.8 \mu\text{g./kg./hr.}$

A sample collected immediately following cannulation under sodium pentobarbital anesthesia indicated that a high rate of secretion occurred in response to operative trauma. The dog was maintained under sodium pentobarbital anesthesia and at 3 hr. after cannulation a second sample was collected or the animal was allowed to recover and reanesthetized after 24 hr. for the collection of a second sample. Analysis showed that the adrenal secretion reached a low and probably steady level and averaged $7.0 \mu\text{g./kg./hr.}$ for 53 dogs (134 to 138).

SECRETION RATE UNDER CONDITIONS OF STIMULATION

A variety of stimuli has been demonstrated to increase by a factor of 5 to 10 the rate of secretion of 17-hydroxycorticosteroids by the dog adrenal. Secretory activity is considered maximal if it equals the rate induced by a large intravenous dose (20 to 40 units) of ACTH. The average rate of secretion of 17-hydroxycorticosteroids was $48 \mu\text{g./kg./hr.}$ for 17 dogs given large doses of ACTH. Under the following variety of circumstances, rate of secretion of 17-hydroxycorticosteroids by the dog adrenal was increased to 25 to $80 \mu\text{g./kg./hr.}$: the surgical trauma associated with adrenal vein cannulation under pentobarbital anesthesia, inhalation of 20 per cent CO_2 under pentobarbital anesthesia, intravenous administration of 0.3 M hydrochloric acid or 1.0 M sodium bicarbonate under pentobarbital anesthesia, exposure to an environmental temperature of -50°C. or of -75°C. (unanesthetized) and immersion in hot water bath with rectal temperatures rising to 42°C. (pentobarbital anesthesia) (42, 134, 135, 138).

Interesting relationships between the duration of the stimulus and the duration of increased secretory activity of the adrenal are revealed by the experiments of Richards and co-workers. Continued exposure (4 hr.) to an atmosphere of 20 per cent carbon dioxide was associated with a persistently

⁷ The reviewers have expressed steroid secretion by the adrenal as $\mu\text{g./kg.}$ of body weight/hr. Our estimates may not correspond precisely to the rates presented in the original reports since we have in a number of instances used approximate body weights of the animals to arrive at our estimates.

high level of adrenal secretion. However, in the instances of exposure to cold and to heat, the level of 17-hydroxycorticosteroid output, initially high, decreased with time to basal levels despite continued exposure. The animals exposed to cold exhibited a typical response to ACTH at the termination of the exposure, which suggests that the cause of the reduction in steroid secretion was not "exhaustion" of the adrenal cortex but rather decreased elaboration of ACTH by the adenohypophysis. A biphasic response in ACTH discharge has been reported to occur in rats exposed to ether, Pitressin or histamine (145) and after operative trauma (23). The phenomenon may represent development of resistance on the part of the animal to the stimulus or the initial excitation and subsequent depression of a neural mechanism concerned with ACTH release. There are as yet no experimental data which favor either hypothesis.

Quantitative estimates based on analysis of adrenal vein blood are now available for the rate of secretion of the adrenal steroids of the rat (17, 149).

PITUITARY REGULATION

The rate of secretion of 17-hydroxycorticosteroids by the adrenal of the hypophysectomized dog under pentobarbital anesthesia in one series was 7.2 $\mu\text{g.}/\text{kg.}/\text{hr.}$ (135) and in another 2.8 $\mu\text{g.}/\text{kg.}/\text{hr.}$ (138). The hypophysectomized dog exhibited no increase in secretion of steroid in response to hyperthermia (135), or to inhalation of 20 per cent carbon dioxide (138). The results establish the role of the pituitary in mediating the increase in secretion of 17-hydroxycorticosteroids which occurs in the intact dog in response to a variety of stimuli.

Farrell, Rauschkolb & Royce (47) have isolated, identified, and quantitated a number of adrenal vein steroids in the dog. Intact and hypophysectomized dogs were bled from the adrenal vein under pentobarbital anesthesia. Hypophysectomy reduced the average rate of secretion in the case of cortisol from 31.6 to 2.4 $\mu\text{g.}/\text{kg.}/\text{hr.}$, 11-desoxy-17-hydroxycorticosterone from 3.7 to 0.27 $\mu\text{g.}/\text{kg.}/\text{hr.}$, 11-desoxycorticosterone from 0.28 to 0.04 $\mu\text{g.}/\text{kg.}/\text{hr.}$ The study demonstrates the marked dependence of the adrenal on the adenohypophysis in regard to secretion of cortisol, corticosterone, 11-desoxy-17-hydroxycorticosterone, 11-desoxycorticosterone, and the relative independence of the adrenal on the adenohypophysis in regard to the secretion of aldosterone. In the rat, hypophysectomy is followed by a greater reduction in adrenal secretion of corticosterone than of aldosterone (149). The renal excretion of aldosterone in patients with panhypopituitarism is normal (97), which indicates that in man, as well as in the dog and the rat, aldosterone secretion is relatively independent of pituitary control.

REGULATION OF ALDOSTERONE SECRETION

The concept of separate control mechanisms of the secretory activities of the adrenal cortex, one concerned with steroids active in carbohydrate metabolism, the other with steroids active in electrolyte metabolism, was

enunciated by Swann in 1940 (157). Recent experimental studies have established the validity of this concept. Evidence based on analysis of adrenal vein blood, cited in a previous section, indicates that aldosterone secretion by the adrenal is relatively independent of the pituitary. However, demonstration of this independence leaves unanswered the question as to the mechanism controlling the rate of secretion of this sodium-retaining factor.

What is the primary initiating event which leads to an alteration in the rate of secretion of aldosterone? Is it the composition of electrolytes, pressure or volume in one or more fluid compartments? And at what site do these primary initiating events act? On the adrenal cortex itself, or on a brain center which secretes an "aldosteronotropic" factor, or on peripheral receptors connected to the brain center by neural pathways? The answers to these questions have an important bearing on the understanding of the homeostatic mechanisms that maintain normality of electrolyte composition and volume of fluid compartments. They also may represent an important basis for the understanding of the aberrations in fluid and electrolyte balance in pathologic conditions characterized by sodium retention and edema.

The question of a brain center which elaborates an "aldosteronotropic" factor has passed the stage of speculation and now has some experimental evidence to support it. The change in fluid composition or volume to which the aldosterone regulating mechanism is responsive is being investigated in a number of laboratories, but there is no unanimity on this point. The question of peripheral receptors of the aldosterone regulating mechanism has not received sufficient attention to warrant more than a speculative statement that veins or atria may have "stretch" or "volume" receptors, or that there may be bodies in the vascular system analogous to the carotid chemoreceptors which respond to changes in sodium or potassium concentration of the extracellular fluid.

Before summarizing the experimental evidence on the regulation of aldosterone secretion, it may be wise to briefly consider the two experimental approaches which are currently employed to gain insight into this important problem. Urinary excretion of aldosterone has been assumed to reflect the rate of secretion of the steroid by the adrenal cortex. The fact that a relatively small fraction of administered aldosterone appears in the urine as biologically active steroid and the estimate of Davis *et al.* (36) that less than one per cent of the aldosterone secreted by the adrenal of the dog finds its way into the urine, should caution investigators in accepting this index of aldosterone secretion without reservation. Obviously of importance in determining the quantity of aldosterone in the urine are the other factors which are responsible for the disappearance of 99 per cent of the steroid. The use of urinary excretion of aldosterone as an index of adrenal secretion rate has the distinct advantage that it does not incur discomfort to the experimental subject.

Analysis of adrenal vein blood is the direct approach to the estimate of rate of secretion of aldosterone. However, the method is not without dis-

advantage since the trauma involved in tapping the adrenal vein and the blood loss probably represent disturbances which in themselves alter the rate of secretion of aldosterone. For these reasons, determinations made in an intact dog may represent maximal or near maximal rates of secretion of aldosterone. Thus, the technic is adapted to reveal factors or experimental conditions which lower the rate of secretion of aldosterone.

Diencephalic center.—Rauschkolb & Farrell (132) have demonstrated that the output of aldosterone (as measured by analysis of adrenal vein blood) is not reduced by decortication or by severing all nervous connections between the head and the trunk in the dog. Ablation experiments suggest that a center in the diencephalon elaborates a humor which is carried in the blood stream to stimulate aldosterone secretion. The precise localization of the center which elaborates an "aldosteronotropic" factor and the isolation of the factor itself are awaited with interest.

Initiating stimuli.—Attention has been directed toward alterations in potassium concentration, sodium concentration, and volume or pressure of body fluids as the primary initiating events leading to a change in the rate of aldosterone secretion.

Potassium deficiency or deoxycorticosterone administration induced a marked reduction in the rate of secretion of aldosterone by the rat adrenal (149). In man, a potassium load has been reported to increase urinary excretion of aldosterone (45). In a study of dog and man, Laragh & Stoerk (94) have concluded that the sodium-retaining activity of urinary extracts can be correlated with an experimentally induced increase in the serum potassium ion concentration, but does not correlate well with reduction in serum sodium ion concentration. "The data support the concept that an increase in the concentration of the serum potassium ion is a stimulus for the secretion of aldosterone" (94).

In contrast to these observations, potassium in excess in the diet or potassium infusion did not induce a significant change in aldosterone secretion by the dog adrenal (142). Rosnagle & Farrell suggest that the discrepancy between their own experimental results in which adrenal secretion of aldosterone was measured, and those of Laragh & Stoerk, in which urinary aldosterone was measured, may be explained on the basis of an effect of potassium on the renal handling of aldosterone, perhaps by decreasing tubular reabsorption of filtered aldosterone. Bartter *et al.* (13) found no correlation between aldosterone excretion and plasma potassium ion concentrations in a series of normal subjects and patients. Lack of uniform results on the effect of potassium remains unexplained.

Deprivation of sodium has been reported to increase the rate of excretion of aldosterone in man (96, 98) and to increase the rate of secretion of the steroid by the adrenal in the dog (142). The deprivation of sodium in man did not significantly alter renal clearance of inulin or the urinary output of 17-hydroxycorticosteroids or 17-ketosteroids (96, 98). The data suggest that the action of sodium restriction is not mediated by ACTH. Further support

for this conclusion is the fact that cortisone did not suppress the elevation of aldosterone excretion in response to sodium restriction and that sodium deprivation induced an increase in aldosterone excretion in a patient with panhypopituitarism (96).

Rosnagle & Farrell (142) found no change in the concentration of serum sodium in dogs deprived of sodium and exhibiting an increase in aldosterone secretion. The authors suggest as do Luetscher & Axelrad (98) that the increased output of aldosterone may be mediated through reduction in extracellular fluid volume. Bartter *et al.* (13) have presented experimental evidence that some function of extracellular fluid volume, and not sodium *per se*, mediates the regulation of aldosterone secretion. In man, expansion of body fluids with water and administration of Pitressin resulted in gain in weight, hyponatremia, and decreased urinary aldosterone excretion; the converse condition, contraction of extracellular fluid volume and hypernatremia was associated with an increase in the rate of excretion of aldosterone.

The findings of Bartter *et al.* (13) that aldosterone excretion may increase in spite of hypernatremia indicates an independence of aldosterone secretion on sodium concentrations which is in contrast to the dependence of anti-diuretic hormone secretion on effective serum osmolality. The authors suggest that a dual feedback mechanism controls sodium and water homeostasis. In response to decreased volume of body fluid, aldosterone secretion is increased with a consequent increase in the concentration of sodium in extracellular fluid; ADH secretion is activated and the water ingested (thirst) is retained and extracellular fluid volume re-established. Some function of the volume increase serves to inhibit aldosterone secretion. No evidence is available as to the mechanism whereby volume changes exert an influence on the adrenal cortex. A number of reviews and symposia on various aspects of the subject of pituitary-adrenocortical physiology have appeared during the past year (31, 83, 118, 129).

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REPRODUCTION^{1,2}

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Expanding world-wide interest in reproduction finds its expression in sustained scientific activity in this field which is attracting increasing numbers of investigators, many hundreds of whom have gathered at international meetings held in 1956, such as the 2nd World Congress on Fertility and Sterility in Naples, mainly devoted to human reproduction, and the 3rd International Congress on Animal Reproduction in Cambridge (England) which dealt with reproductive physiology, pathology, and artificial insemination of farm animals (1). The proceedings of some of the meetings held in 1955, containing much that is pertinent to reproduction, have now become available, among them those of the Laurentian Hormone Conference (2), 5th International Planned Parenthood Conference (3), and the Symposium of the German Society for Endocrinology (4). Major recent publications wholly or partly concerned with reproduction are: a monograph on "Fertilization" (5) which surveys the field in a comprehensive and up to date manner; a book on comparative aspects of vertebrate development (6), and a treatise on parthenogenesis and polyploidy in mammalian development (7). A new journal has appeared which aims at publications concerned with breeding, reproductive disorders, and the insemination of farm animals (8).

Whilst scientific endeavour embraces problems ranging freely from the mechanism of gestation in a little-known viviparous toad to the chemical make-up of the early embryo and its environment, and from the intricacies of sperm metabolism to sex differentiation, the world at large is (apart from a growing concern over radiation dangers) primarily preoccupied with but two aspects of reproduction: namely, fertility and sterility, inasmuch as they affect man and animals subservient to mankind. Something akin to a perennial tug-of-war goes on: it is sometimes difficult to make out whether more effort is spent on combating sterility and promoting fertility or curtailing seemingly boundless fertility and inducing voluntary sterility. The world over, sociologists, economists, geopoliticians and humanity's spiritual leaders, debate and make pronouncements, not always concordantly, on these perplexing issues. Nothing is more natural than that biological and medical research should also be directed towards the solution of the many-faceted problems; hence the search for and screening of potential ovulation or implantation-inhibiting factors (9, 10), and sperm-immobilizing agents (11, 12), surveys on periodicity in animal fecundity (13), season-dependent infertility of

¹ The survey of literature pertaining to this review was completed in May, 1957.

² The following abbreviations are used in this chapter: PMS (Pregnant mare's serum); PAS (periodic acid—Schiff reaction); ATPase (adenosine-triphosphatase).

farm animals (14), effects of temperature on fertility (15, 16), accumulation of statistics on human fertility (17, 18) and sterility (19). Regardless of whether and when these investigations will culminate in a safe yet simple method of progeny control, the acceptance of which might rearrange the world's population structure so as to keep it more in line with the best that animal breeders can achieve, much fundamental information will accrue and deeper insight will have been gained into several as yet obscure phases of reproduction.

SEX RATIO REVERSAL, AND DETERMINATION

If the power to influence at will the sex ratio of progeny, at any rate for veterinary if not for medical purposes, is not yet ours, this is not so for lack of scientific endeavour.

An attempt was made to bring about the separation of "male" and "female" bull spermatozoa by fractionating semen in the counter-streaming centrifuge (20); when the heavier sperm fraction was used to inseminate 24 cows, 12 became pregnant, giving birth to 11 live calves, all of them male; in view of this result, the outcome of similar large-scale field trials will be eagerly awaited. It is also held possible that a practical system of controlling sex in animals might be established by selecting sires according to their pH values in blood, "acid" lines being predisposed towards an excess of female, and "alkali" lines to an excess of male progeny (21).

A rare example of a spontaneous sex reversal from male to female in domestic birds has been described, and the cause of it traced to the growth of a Sertoli cells tumor endowed with estrogenic activity (22). In the crustacean, *Pandalus borealis*, which changes from male to female at moult, both the testis and ovary are present during the male phase but the activity of the latter is inhibited by a hormone produced in the sinus gland of the eyestalk; when, however, towards the end of the male phase the secretion of the hormone ceases, sex reversal takes place (23). In the toad, *Xenopus laevis*, sex inversion, also from male to female, has been successfully performed with estradiol (24).

The fact that sex can be diagnosed from differences in the structure of chromatin present in somatic cells has been made use of for anatomical, clinical, and forensic purposes. Methods have been developed for accurate sex determination from blood films in man (25 to 29), dog (30) and rabbit (31) and from epithelial cells of human skin (32); the sex-difference in skin-chromatin was shown to persist for a considerable time after death. In pregnant women, correct prenatal sex diagnosis has been made by "cellular sexing" of placental endothelium (33) and of amniotic fluid removed from the amniotic sac by direct aspiration (34, 35, 36). Similar attempts, however, in cattle and sheep were unsuccessful (37). In rats on the other hand, ameloblasts from the dental germ of one-day old animals have been utilized successfully for the purpose of sex differentiation (38).

GONADOTROPHINS

The ceaseless flow of papers on the correct assessment of gonadotrophic activity in the pituitary gland, placenta, blood, and urine, not only indicates the growing need for such methods in physiology and medicine but reflects also the present rather unsatisfactory position in bioassay techniques, due partly at least, to the lack of simple yet reliable means for the separation of the two main gonadotrophins, the follicle-stimulating hormone (FSH) and the interstitial-cell stimulating hormone (ICSH).

When tests are based on ovarian hyperemia in rats or spermiation in frogs, the potency of a gonadotrophin preparation can vary according to the nature of the solvent in which it was administered (39, 40). Environmental conditions prevailing among the test-animals before and during the bioassay constitute another factor capable of influencing the results, and that is why good response from test-animals and a reproducible dose-response curve can be expected only if standard conditions are strictly observed (41 to 46). The progress, however, in the field of techniques for bioassay of gonadotrophins continues, and there is no lack of attempts at separating and determining accurately FSH and ICSH potencies (47 to 51).

In young deer, the highest gonadotrophic activity in the pituitary glands was displayed during summer, in the adult animal, however, in the autumn (52). In hamster, no significant difference was observed in the gonadotrophic activity as between winter and summer (53). In rats, injections of testosterone resulted in an increase of FSH activity of the pituitary gland, while estradiol produced the opposite effect (54). In hypophysectomised male rats growth of accessory organs was enhanced by a nongonadotrophic pituitary hormone (55). In castrated mice growth of intraocular grafts of neonatal gonadal tissue was stimulated by chorionic gonadotrophin (56). In the pig, contrary to earlier assumptions, the gonadotrophin content of the pituitary gland was found to be particularly low at puberty (57). The FSH of the pig pituitary is now available in a state of considerable purity (58); FSH has also been purified from sheep pituitaries to an extent where it is free from both the adrenocorticotrophic and thyreotrophic hormones (59). In dogs, the male and female pituitaries showed approximately the same gonadotrophin content (60). In mares which had been covered by jackasses the level of blood gonadotrophin during pregnancy was *nil*, in striking contrast to those mares which had been mated to stallions (61). As regards, however, the gonadotrophic activity of pregnant mares' serum (PMS), there is increasing evidence that this may be due to more than one hormonally active component (62, 63, 64). In rabbits, higher gonadotrophic activity was observed in the blood from mature than from immature females (65); in this species the ability of injected chorionic gonadotrophin to produce hemorrhagic follicles in ovaries can be suppressed by the injection of a substance present in colostrum (66). Human placental gonadotrophin is endowed with ability to stimulate the development of testicular interstitial tissue (67).

In the trout, maturation of infantile testes was achieved by administration of salmon pituitary gonadotrophin (68).

As regards the mechanism by means of which gonadotrophins are released from the pituitary gland, the concept of humoral adrenergic transmission from the hypothalamus to the anterior hypophysis failed to receive support (69). There have been, on the other hand, more observations on the involutionary changes in testes and accessory organs, resulting from hypothalamic lesions (70) and on the action of the "neurogenic" stimulus which induces the release of gonadotrophins from the hypophysis (71); further evidence is available that the gonadotrophins are localized in the "basophiles" of the anterior hypophysis (72).

THE MALE

Androgens.—Additional evidence has been obtained that testosterone is a secretory product of the human testis by the demonstration that the hormone is present in the blood from the spermatic vein but is absent in arterial blood (73). Following the earlier finding that dehydroepiandrosterone is present in the human peripheral blood plasma, androsterone has now been isolated from the same source (74). The reduction of androstane-3,17-dione to androsterone, epiandrosterone, androstane-17 β -ol-3-one, and androstane-3 α ,17 β -diol takes place in homogenates from guinea-pig liver (75), and the conversion of [¹⁴C]-testosterone to [¹⁴C]-estradiol-17 β was found to occur in slices of the human ovary, and also in the stallion testis, an organ notoriously rich in estrogens (76). Antagonistic effects of testosterone and estradiol have been studied in pullets (77), rats (78) and mice (79); the inhibition of the body growth of hypophysectomised immature female rats by estradiol was partly prevented by the simultaneous administration of testosterone, and the inhibition of the estrogen-induced vaginal cornification in mice was successfully brought about, by a local application of testosterone. The synergistic effect of prolactin on the response of accessory organs to testosterone was studied in rats (80).

An interesting example of the renotropic potency of androgens has been provided by the observation that inhalation of small amounts of chloroform by mice induces extensive necrosis in the renal tubules of male but not female animals. Female mice, however, can be rendered susceptible to necrosis by injections of androgen, while male mice become insensitive as a result of castration (81). When, however, increasing doses of androgen are administered to castrated mice, the amount of damage rises proportionally (82). Renal β -glucuronidase activity in male mice which is assumed to be under androgenic control, can be greatly increased by gonadotrophin administration (83). New observations have also been reported on the effect of androgens on the activity of transaminases in muscle (84) and on the level of ergothioneine in blood (85).

Spermatogenesis.—Whilst histological methods continue to provide much valuable information on testicular morphology in general (86) and on the

gametogenic function of the testes in particular (87), new techniques, including the application of radioactive tracers, have now found a permanent place among the tools available for experimental purposes.

Radioactive tracers have shown themselves particularly useful in the elucidation of the complex time relationship between spermatocytogenesis, spermiogenesis, and sperm maturation. In the boar, after feeding of [S^{35}]-methionine, the maximum of labelling with radioactive sulphur was observed in the seminal plasma within a few days, but in spermatozoa only after three to six weeks (88). In the bull, the excretion of P^{32} in semen reached a maximum at 15 to 19 days after feeding of phosphate, and even then radioactivity appeared to be confined mainly to the seminal plasma (89). In ram, experiments with P^{32} showed that some 40 days are required for spermatogenesis to run its course in the testis, and that some additional 20 days are needed by the spermatozoa to mature in the epididymis. The exact length, however, of these two processes, depends largely on environmental factors, including light for example; with extending daylight, the epididymal reserve of ram spermatozoa is known to decline markedly (90, 91, 92). In the mouse, autoradiographic experiments with [C^{14}]-adenine have shown that in the testis 12 per cent of spermatogonia are labelled one day after injection of adenine, and 63 per cent after 10 days, while in spermatozoa labelling appears at 25 days in the testis, and at 32 days in the epididymis. At the same time it was noticed that the rate at which [C^{14}]-adenine is incorporated into deoxyribonucleic acid is slower in spermatogonia than in somatic cells, but that ribonucleic acid is formed more quickly. This may be taken as one more indication that ribonucleic acid actually acts as a precursor in the process of deoxyribonucleic acid synthesis by the sperm cells (93, 94). Radioactive tracer techniques have also been applied successfully to the study of spermatogenesis in *Drosophila* (95, 96).

Another valuable experimental approach is the technique of irradiation. In *Drosophila*, fast neutrons have been used to induce chromosome breaks in the male gametes (97). In insects, radiation which resulted in sterilization helped to control insect pests which infest cereals (98). In the mouse, x-rays were applied to determine the mutagenic sensitivity of the immature sperm cells at various stages of development (99, 100, 101).

Recent results obtained by cytological techniques provide fresh evidence for the development of the acrosome from the Golgi apparatus in the toad (102) and the identification in the chromosomes of dividing mammalian spermatocytes of a central dense structure consisting of a pair of fibrils (103). Studies on the hormonal control of spermatogenesis include experiments on the effect of cortisol on spermiogenic activity in hypophysectomised rats (104) and a demonstration that in the frog, spermatogonia are less sensitive to gonadotrophin during the resting period than during the spermatogenic period (105).

Semen.—Recent improvements in semen collection methods are largely due to the technique of electro-ejaculation, specially in application to bulls

(106, 107, 108) and rams (109). Better preservation of semen was achieved by further developments in the glycerol-freezing technique (110 to 113) and in the use of artificial diluents (114 to 122). Interest in artificial insemination as a means for livestock improvement is turning gradually from cattle and sheep to other species, particularly the pig (123, 124, 125). In the horse, success was claimed after insemination of epididymal semen which had been stored for a month at -79° in a diluent composed of milk and glycerol (126). In the guinea-pig, pregnancy was set up by intraperitoneal injection of spermatozoa (127). In the cock, there is a diurnal rhythm in semen yields which determines the number of spermatozoa available for insemination (128).

The phenomenon of induced fluorescence resulting from treatment with certain dyes such as acridine orange has been utilized for the study of sperm structure, and for the purpose of differentiation between live and dead spermatozoa (129). Among the other differential stains, nigrosin-eosin was subjected to critical evaluation (130). In the boar, the proportion of spermatozoa containing the "cytoplasmic bead" (residual cytoplasm) was greater in the *vas deferens* than in semen as ejaculated and it is possible that the removal of the cytoplasmic bead in boar semen is normally brought about by the action of accessory secretions (131). Further information has also been provided on the morphological changes which take place in human spermatozoa after ejaculation (132). Two new methods for measuring the velocity of sperm movements have been described (133, 134); the mean speed of spermatozoa in fifteen samples of bovine semen was calculated to be 4.23 mm./min. (134). A definite correlation exists between motility and the sedimentation rate of ram spermatozoa under storage conditions *in vitro* (135).

The antigenic properties of semen were studied in guinea-pig (136) and man (137); in human semen the dominant antigenic material appears to be derived from the seminal plasma and not from spermatozoa. The sperm "anti-agglutinin factor" has been further purified and found to be a protein complex which contains a tocopherol-like substance, a carbohydrate, and a sulphuric acid residue in the prosthetic group (138). The anti-agglutinin factor also occurs in the female reproductive tract, e.g., in ovarian follicles, tubal secretion, and cervical mucus (139).

Biochemical research on semen continues to expand in several directions; one of these concerns the role of mineral constituents in semen, particularly that of potassium ions which appear to play an important function in eliciting sperm movements (140 to 143). Much attention is also focussed on zinc, which is present in high concentrations in mammalian semen, particularly of man (144); a characteristic injury to the testes can develop in nutritional zinc deficiency (145). In this connection special significance attaches to the finding that unlike zinc salts, those of cadmium are extremely toxic to the testes but the toxicity can be prevented completely, in the rat at any rate, by a simultaneous administration of zinc (146, 147, 148). Although in in-

vertebrates the zinc content of sperm is, on the whole, much lower, it possesses great physiological significance. This follows, for instance, from reports that starfish spermatozoa suspended in seawater, become suddenly motile on addition of L-histidine, the effect coinciding with a release of a considerable amount of zinc from the sperm cells into the surrounding medium (149, 150, 151). Cytochemical analyses of starfish spermatozoa point to the middle piece as the chief site of zinc accumulation (150). There is apparently a link between zinc and the so-called dilution effect, a phenomenon which is particularly characteristic of sea urchin sperm, and which manifests itself as a sudden spurt of motility and respiratory activity in sea-urchin semen after dilution with sea water (152, 153). For the time being, however, the mechanism of the dilution effect remains rather obscure; it involves probably, apart from zinc, some other metals, and a variety of additional factors, including the hydrogen ion concentration (154, 155) as well as certain chemical activators and inhibitors (156, 157).

Another line of research vigorously pursued in sperm biochemistry concerns the metabolism of certain organic substances such as fructose, inositol, citric acid, ergothioneine, and glycerylphosphorylcholine in mammalian seminal plasma. Experiments with the "semen perfusion apparatus" designed to keep bull spermatozoa motile for considerable periods of time, have shown that sperm motility can be maintained at the highest level when fructose is present in the perfusion fluid and oxygen supplied at the same time; in the absence of both fructose and oxygen, the spermatozoa are quickly immobilized but recover, at least partly, on the subsequent addition of either fructose or oxygen (158, 159). The importance of fructolysis as an energy-supplying process was again confirmed by new measurements of the rate of fructolysis (160, 161) and of heat production in bull semen (162). More work was done on the relation between pyruvate metabolism and quality of semen (163). The close dependence of the fructose level in semen upon androgenic activity was demonstrated in bulls (164) and also in men (165).

The level of inositol has been determined in the semen of various animal species (166); in man it was found to increase, together with fructose, in response to testosterone injections (167). The association of inositol with reproductive processes in the widest sense is strengthened by the finding that this hexitol is not restricted to semen and foetal fluids but occurs, in extraordinarily high concentrations, also in plant-pollen (168). The observation that boar and stallion semen contain ergothioneine has been confirmed and extended to other species (88, 169). Further study is needed to test the possibility that apart from ergothioneine, there are in seminal plasma some other sulphhydryl compounds, and that the SH-content of semen may in some way be related to fertility (170, 171).

The phosphorus compounds of semen, recently studied, include sperm nucleic acid, particularly as regards the phosphorus/arginine ratio in nucleoprotein (172 to 175); sperm phospholipides and their role in aerobic metabo-

lism (176); inorganic pyrophosphate which had been identified in the contents of the ejaculatory duct of the hawk-moth, and shown to be transferred from the male to the female insect during copulation (177, 178); and glycerylphosphorylcholine which in several mammalian species such as the bull, ram, goat, boar, and stallion, accounts for the bulk of the acid-soluble phosphorus, choline, and bound glycerol in seminal plasma (179). Glycerylphosphorylcholine itself is not metabolised by spermatozoa but glycerol is readily oxidized, yielding lactic acid as a by-product (180, 181). In that respect glycerol shares the properties of two other substrates, namely, dihydroxyacetone and sorbitol; fructose is the primary oxidation product of sorbitol (182). It should be emphasized, however, that although glycerol is oxidized by spermatozoa it is highly improbable that its preserving effect on frozen semen is dependent on the metabolic activity of spermatozoa. The concentrations of glycerol which must be added to semen for freezing purposes are much higher than those required for sperm respiration. The oxygen uptake of glycerol-frozen semen declines rapidly on thawing (183).

Glycine, another substance introduced recently into semen diluents (117, 184, 185), is metabolised by bovine spermatozoa to carbon dioxide, glyoxylic acid, and formic acid; the latter was found to occur also in fresh bull semen (186). Bovine spermatozoa also are capable of incorporating amino-acids from the extracellular medium into intracellular proteins (187). Other problems relating to sperm metabolism and motility include the influence of metals and chelating agents (188), phosphate and chloride (189) and vesicular secretion (190). Electrophoresis of bull seminal plasma indicates the presence of at least seven distinct protein fractions (191). Seminal proteins comprise a variety of enzymes; those recently investigated are glycosidases (192, 193), dopa oxidase (194), choline esterase (195) and adenosine triphosphatase (196).

Male accessory organs.—In various mammals including man, ram, bull, boar, and guinea pig, the seminal vesicle is the chief producer of seminal fructose. The formation of fructose depends on the presence of the male sex hormone; it is abolished by castration or hypophysectomy, and restored promptly by testosterone injections (169, 197). There is some evidence that in the bull seminal vesicles the glycogen-laden cells of the inner layer of the alveoli are actually responsible for the secretion of fructose (198). In the guinea pig, under conditions *in vivo*, the secretion of fructose is accompanied by a characteristic redistribution of water and ionic movements in the vesicular tissue; the normal ionic concentrations of sodium, potassium and chloride in the seminal vesicle mucosa can only be maintained for so long as either respiratory or glycolytic metabolic energy is available to the tissue (199). When glucose is added to slices or homogenates of sheep seminal vesicles fructose and sorbitol are produced (200, 201, 202). In the rat, the seminal vesicles lack the ability to produce fructose, but secrete citric acid and glycerylphosphorylcholine (179, 203); the latter is probably derived from phospholipides.

A characteristic feature of the coagulating gland both in the rat and in the guinea pig, is the secretion of vesiculase, an enzyme which is responsible for the coagulation of the seminal vesicle secretion and thus leads to the formation of the "vaginal plug." Vesiculase has been purified and separated from another highly active enzyme in the coagulating gland, which hydrolyses certain arginine esters (204). In bilaterally adrenalectomised male guinea pigs, the bladder is often filled with characteristic "lumps" of coagulated material. This, in all probability, is formed as the result of a peculiar disturbance in the normal ejaculatory process, during which the secretions of the coagulating glands and seminal vesicles are ejected into the bladder, where they undergo coagulation (205). Another peculiarity of the coagulating gland secretion in the guinea pig is the presence of a substance resembling in its action certain parasympathomimetic drugs, which when injected intravenously or intramuscularly produced severe toxic effects and death (206).

Further studies have been reported on the effects of castration and hormonal treatment on the prostate gland in man (207) and in rat (208); on the mechanism which leads to the accumulation in the gland of extraordinarily large quantities of zinc (209, 210), and on the structure, composition, and origin of prostatic *corpora amylacea* (211, 212). The arginine-ester hydrolysing enzyme which is present in the prostate gland of various species was shown to be very active in the prostatic fluid of the dog (213). The secretion of the epididymis in bull and boar is distinguished by a high concentration of glycerylphosphorylcholine (179). In the Cowper's gland of goat and ram, an "egg-yolk coagulating enzyme" has been demonstrated (214). In the cock, the vascular bodies in the cloaca, which histologically resemble the bulbo-urethral glands of mammals, have been shown to produce a secretion which abounds in mucopolysaccharides (215).

THE FEMALE

Oogenesis and the ovum.—The problem of postnatal (216) and postpubertal (217) oogenesis in the mammalian ovary continues to attract a great deal of attention. Most of the evidence available from recent experiments on mice, rats, and rabbits dismisses the possibility that neoformation of oocytes can occur either in ovarian grafts (218, 219) or cultures of ovarian tissue (220), or for that matter, in the ovary of the postpubertal mammal as a whole (217). Those oocytes that have been observed in surviving ovarian grafts are most likely the remains of a stock of primordial oocytes present in the ovarian tissue at the time of transplantation. The survival of ovarian grafts depends on a large number of factors (221). In rats the survival of grafts for a given length of time has been shown to be influenced strongly by spaying and genetic diversity (222). Grafts derived from ovarian tissue which has been treated with glycerol and stored at -79° , are hormonally active as assessed by the appearance of vaginal cornification, but contain comparatively few oocytes (223, 224). Nevertheless, those oocytes which have survived freezing and thawing are capable of maturing and even undergoing

ovulation (225); it remains to be seen, however, whether they are also capable of normal fertilization and development. An approach was made to the problem of orthotopic grafting of ovarian tissue as a means of restoring fertility, by placing the tissue in the ovarian sites of genetically distinguishable recipients which had been previously ovariectomized or sterilized by x-ray treatment (226, 227, 228).

Among the techniques used in studies of ova, particularly those of mammals, the method of egg-transplantation continues to yield interesting results. Its potentialities have been explored in several species, including the mouse (229), rabbit (230, 231), and sheep (232). Recent advances in ovum morphology concern the structural damage to rat ova, induced by x-rays (233), and new interesting data on the presence of microelements in the cortical zone of the Triton egg (234). Heat and cold have been used as means of inducing heteroploidy in fish eggs (235). Annelid eggs have been analysed chemically and a number of guanidine derivatives identified as their constituents (236). Sea-urchin ova change their rate of cleavage in response to certain substances present in extracts from ovaries (237). The effect on spermatozoa of substances present in sea urchin eggs is associated with characteristic changes in the acrosomal region (238).

Estrogens and progestogens.—Progress was made in techniques for the detection and assay of estrogens, particularly in the urine (239 to 245). Both estrone and estradiol-17 α have been isolated from cow's urine, together with androstane-3 β :17 α -diol (246). An estrone-like steroid is present in the blood and ovary of laying hens (247). A ketonic chromogen, containing probably 16-hydroxyestrone as the principal component, has been isolated from the urine of pregnant women (248). In women, urinary excretion of estrogen continued even after oophorectomy and adrenalectomy (249). In men, one of the changes observed in the urine after administration of estrogen was a markedly diminished output of androsterone (250). Tissue slices and homogenates have been used for experiments on the incorporation of [14 C]-acetate into estradiol (251) and on the conversion of estrone and estriol into estradiol (252). Further experiments have been reported on the activating effect of estriol on isocitric dehydrogenase (253), and of estradiol on the rate of serine incorporation into purine bases and other components of uterine tissues (254).

Estrogenic effects, recently studied, include the uterotrophic action of ethinylandrostanediol (255, 256); the ability of natural and synthetic estrogens to cause cultures of vaginal epithelial cells to proliferate, stratify and keratinize (257); increased mitotic activity in mouse tissues, following a single injection of estradiol benzoate (258); reduced estrogenic activity of estradiol and estrone in rats, following hepatectomy (259); maintenance of corpus luteum by low doses of estrogens in rabbits (260); metabolic and growth-promoting action of estriol on the uterus and vagina of the guinea pig and rabbit (261); mucous metaplasia of the prostate gland in immature guinea pigs following administration of stilbestrol (262); and an inhibitory

effect of estradiol on the fixation of radioactive sulphur in the vaginal epithelium (263). Much thought and study is being devoted to the complex problem of the estrogen-progesterone interaction (264). From studies on sheep (265, 266) it would appear that while the vaginal cornification at estrus is the result of estrogen stimulation per se, the normal manifestation of leucocytic invasion and the changes in the viscosity of mucus probably depend on an interaction between estrogen and progesterone. Moreover, the sensitivity of the spayed ewe to an estrus-inducing dose of estrogen increases with increasing duration of progesterone pretreatment (267).

The introduction of paper chromatography into steroid analysis has helped to establish new values on the actual content of progesterone in blood and tissues. The average content of progesterone in the human placenta was shown to rise from 33 $\mu\text{g.}$ in the second, to 713 $\mu\text{g.}$ in the last month of pregnancy (268). In the mare the placenta contains several hundred $\mu\text{g.}$ progesterone/Kg., in addition to 20 β -hydroxypregn-4en-3-one (269, 270). The blood progesterone level is considerably lower in domestic animals (270) than in women (271). In laying hens progesterone has been demonstrated in the ovaries (272). Metabolic studies on progesterone and related compounds include recovery experiments of urinary pregnanediol in men and women (273, 274); identification of pregnanetriol in the urine of a boy suffering from adrenal hyperplasia (275); experiments on the fate of progesterone in rats (276 to 279); and the identification of two new substituted androstane derivatives in pregnant mares' urine (280). Among biological effects of progesterone recently described was the stimulation of the secretory function of the toad oviduct (281); maintenance of pregnancy in rats (282); effectiveness in the treatment of habitual abortion in women (283, 284); inhibition of follicular growth and ovulation in cows (285); inhibition of mouse egg development *in vitro* but not *in vivo* (286, 287); and an *in vitro* acceleration of mitochondrial adenosinetriphosphatase activity in rats (288).

As the result of continued efforts, several new synthetic compounds have been prepared and shown to possess marked luteoid activity (289 to 293). Some of these compounds appear to be particularly effective in inhibiting ovulation and could thus be considered as "antifertility factors" (9, 294, 295). Certain other progesterone derivatives have been found to have marked antiluteinizing activity (296). A simple and rapid enzyme test for assaying the potency of progesterone and related steroids has been worked out, based on the observation that the progestational response of the rabbit uterus is closely correlated with the activity of carbonic anhydrase in the endometrium (297).

Relaxin.—Progress has been reported on the purification of relaxin (298). A characteristic pattern of changing sensitivity to relaxin exists in guinea pigs; a rapid increase of sensitivity in the animals used for relaxin assay was shown to be followed by a period of constancy which then passes into a state of increasing refractoriness (299).

Estrus.—Recent studies include a number of new observations on the

modifying influence of various environmental factors and hormones on the estrous cycle. In the mouse, estrus was studied in relation to age (300) and the external stimulus associated with the presence of the male (301). Mouse ovaries and uterus regress after the animals have been rendered "anosmic" by removal of the olfactory bulbs (302). In the rat, changes which occur in the vaginal epithelium during estrus, have been followed up by means of the alkaline phosphatase technique (303). Studies on estrus in the rabbit deal with the effect of chlorpromazine (304) and ovariectomy (305); some of the does which were ovariectomized after having had litters and lactated, continued to exhibit estrous behaviour for up to seven days after the removal of the ovaries. In the field vole, *Microtus agrestis*, which resembles the rabbit in having prolonged periods of estrus during which ovulation can be induced either by copulation or by gonadotrophin injections, the sexual "receptivity" at estrus varies according to breeding conditions, e.g., whether or not the sexes are kept together (306, 307). In the ferret, some more work has been reported on the estrous response to prolonged illumination, and sympathectomy was shown to delay this particular kind of response (308). In the guinea pig, radioactive sulphur injected during estrus appears in marked concentrations in the cervical secretion (309). In sows, gonadotrophin-induced estrus was shown to occur in 76 per cent of animals injected with pregnant mares' serum between the 20th and 39th days of lactation, and in 86 per cent when the injection was given between the 40th and 50th days (310). In ewes the "heat" periods which take place during pregnancy were anovulatory (311). In monkeys some experiments were carried out on the inhibitory effect of reserpine on menstruation (312) and on changes in the iodine-level of blood during the menstrual cycle and pregnancy (267, 313). An interesting study was done on the ovulation in the badger. In South-West England, the female badger, having reached maturity during its second year, ovulates for the first time either in the early months of the year or a few months later. If ovulation has taken place during the early months then it is followed by a considerable delay, up to nine months, of implantation, during which time three further ovulations can occur (314). Gonadotrophin-induced ovulation was studied in hens, a dose of 0.5 mg. luteinizing hormone per bird being effective in 85 per cent of the animals (315).

Fertilization.—A great deal of research was concentrated upon the morphological and biochemical changes which accompany fertilization in the mammalian and invertebrate eggs and on the influence of exogenous factors on processes leading to ovulation and fertilization. Several investigators studied the process of fertilization in laboratory rodents, the rat, mouse, and hamster (316 to 322). In these three species the spermatozoon penetrates the zona pellucida relatively rapidly and the sperm head becomes promptly attached to the surface of the vitellus. As a result of this attachment a block to polyspermy develops in the rat and mouse, but not apparently in the hamster. A more detailed description is now available of the cleavage stages in the hamster egg (321, 323); it is known that in this animal ovulation

takes place some four hrs. after mating, with blastocyst formation at the 8-cell stage. The majority of hamster eggs are fertilized in the region of the middle and caudal third of the tubal ampulla; it is interesting to note that some 80 per cent of hamster eggs are prone to spontaneous activation. The remarkable resistance of the zona pellucida of rabbit, rat, and hamster eggs to various hydrolytic enzymes (324) makes very desirable further studies on the chemical nature of this peculiar structure. There are indications that the zona pellucida may be the source of mammalian fertilizin (325).

Intrafollicular *in vivo* cleavage of the human ovum without any previous material contact with spermatozoa was observed in three ova out of 400 human follicular and tubal ova examined; one of these ova was at the two-cell, one at the four-cell, and one at the blastula stage (325).

Much interest is shown in the subtle mechanism whereby, in laboratory animals, extraneous factors such as changes in environmental temperature can affect the mating behaviour, timing of mating in relation to estrus, ovulation, and sperm entry (319, 320, 327, 328), especially as regards polyspermy and block to polyspermy. There is some evidence that differences in the incidence of polyspermy in relation to normal or delayed matings may also depend upon the rat strain (329).

Experimental material being both more readily accessible and abundant, many notable contributions to the problem of fertilization continue to be made with the eggs of lower organisms. A remarkable electron micrograph of a sea-urchin egg at three min. after fertilization is now available (330). More is also known about the ultrastructure of the Golgi element (dictyosome) in sea urchin oogenesis, owing to electron microscopy (331); differential centrifuging makes possible to stratify the dictyosome within the eggs. There are morphological studies on the sperm-egg relationship in Holothurians as regards the acrosomal reaction leading to the production of a filamentous structure, the so-called acrosome filament which enters the egg intact and remains largely unchanged until well inside the egg proper (332). Considerable interest attaches to the report according to which blue, but not red, light is capable of abolishing the peculiar movement of fish eggs (333).

The nature of the sperm-agglutinating factor cytofertilizin and its function were the object of several studies (334, 335). A cytofertilizing preparation has been obtained free from jelly, from sea urchin eggs (336); it is distinct from jelly fertilizin, acts upon membrane formation and cleavage and enhances the fertilization rate; calcium favours the release of cytofertilizin. Cytochemical studies on fertilization and first mitosis indicate the presence of polysaccharide in the first mitotic figure (337); the yolk sac granules giving a more intense PAS reaction after than before, fertilization. A mucopolysaccharide with ester-bound sulphate was detected in the cortex of the unfertilized sea urchin egg and in the hyaline layer of the fertilized eggs (338). The dynamics of early echinoderm development were studied by following mitotic cycles with the aid of phase contrast microscopy and correlating them with respiratory measurements (339); the most marked increase in the res-

piratory rate was linked with the maximum development of resting phases, the rate being highest in prophase and decreasing through meta- and anaphase to a minimum at telophase. A review surveys critically data concerned with the phenomenon of increased O_2 -uptake by the sea urchin eggs after fertilization (340). Attempts are being made to define quantitatively the changes which occur in the free phospholipides of *Arbacia* eggs at fertilization (341); apparently a decrease in free phospholipide content during the first few minutes is followed by a recovery to the level existing in unfertilized eggs.

The effect of 2 high-potential indicators, porphyrexid and porphyrindin, on the sea urchin egg was to block fertilization, even at a concentration 5×10^{-6} M (342); with reduced glutathione it was possible to abolish the action of these indicators: it is inferred that a reduction of the S—S bond may be involved in cytoplasmic maturation. The surface of oocytes and freshly fertilized eggs was less sensitive to the oxidizing agents than that of more mature fertilized eggs. Measurements on the rigidity of the cell membrane in the unfertilized sea urchin egg, done with the "cell elastimeter," indicate that the membrane rigidity increases following treatment with dipropanol but is lowered by detergents (343). Observations were made on the extent of folding of the surface in sea urchin eggs: after fertilization there was a marked increase (344). Electrical properties of starfish eggs at fertilization were studied by a method making use of 2 micro-electrodes inserted below the egg surface (345); upon insemination of the eggs the membrane potential first decreased, then increased, reaching finally a steady value, greater than that of unfertilized eggs. Within the first few minutes after fertilization of *Arbacia* eggs there is a release of K ions; a reduction of the rate of release, followed by another increase, has been observed (346).

There is a whole set of problems related to the fate of sperm in the female reproductive tract, which continue to exert the minds of investigators, *viz.* the mode of sperm progress towards the site of fertilization, (whether and to what extent spontaneous or assisted by uterine contractions); the location of the actual site of fertilization in different species; what, if any, modifying changes (capacitation) take place in spermatozoa during their uterine sojourn; and above all else, how is the bulk of the sperm finally disposed of, after a tiny minority have fulfilled their destiny by entry into the awaiting ova. Hence the studies on sperm transport (347); sperm capacitation and its abolition by seminal plasma (348); the ultimate removal of sperm from the female reproductive tract, which is variously attributed to digestive processes in the uterine lumen, or to engulfment of redundant sperm by phagocytes, with a preceding leucocytic invasion of the uterus (mouse, rat), perhaps brought on mechanically (by uterine distension) rather than by chemical stimulation emanating from sperm; earlier claims whereby sperm would actively penetrate the uterine wall being on the whole discounted as artifacts arising from the handling of tissues for histological examination (349 to 352).

Uterus and placenta; composition, metabolism, function.—Data steadily accumulate concerning changes in the chemical make-up of the uterus in relation to uterine function. Thus, for instance, the collagen content of the rat uterus spectacularly increases in the course of pregnancy, whereas a precipitous loss of uterine collagen marks the postpartum or postcastrate involution (353, 354). On the other hand, no clear-cut relationship seems to exist between uterine collagen and the estrous cycle, though some slight increase can be achieved by stimulation with ovarian hormones (355). New analytical data on the mineral content (Na, K, Cl) of the human uterus were reported (356). The glycogen content in the rat myometrium (357) and human endometrium (358) was studied in relation to the cycle and pregnancy. An actomyosin-like protein extracted from pig and rabbit myometrium was found to possess ATPase activity (359). Esterase activity was demonstrated histochemically in the pregnant mouse uterus, the maternal placenta, myometrial gland, and endometrium being highly active, foetal placenta less, and the nonpregnant uterus moderately active (360). Characteristic cyclic fluctuations in β -glucuronidase, sulphatase, and esterase activity were described in the rat uterus (361).

A treatise on endometrial biopsies in cattle is a source of information on the histology and histochemistry of the cow uterus in relation to the sex cycle (362). A morphological study of the terminal nervous system in the cervico-vaginal region of the rabbit points to great complexity and possible hormonal dependence of the nerve endings upon the corpus luteum hormone (363). It is stated that the spontaneous electrical activity and responsiveness to electric stimuli in the rat uterus depends on the availability of estrogens; gonadectomy abolishes uterine responsiveness (364). The oxygen tension in the rabbit uterus was found to equal that of the peritoneal cavity (365). The mechanism of secretion in the rabbit oviduct was studied in relation to endocrine factors, the rate of secretion being high in the estrogenic phase and low in pregnancy (366, 367).

Metabolic changes in the rat uterus induced by treatment with adrenaline or growth hormone became manifest chiefly as an inhibition of endogenous respiration, being very prompt in response to adrenaline, but delayed in response to growth hormone (368 to 371). Respiratory and glycolysis quotients were established for infantile mouse uteri; respiration and anaerobic glycolysis were distinctly enhanced by estradiol treatment, and less markedly by administration of progesterone. The Q_{O_2} in the uteri of adult mice was -5.2 in dioestrus but -8.3 in estrus (372). Characteristic responses of the rat uterus to estrogen administration, such as increase in nucleic acid, phospholipide and acid-soluble P, protein-N, were all abolished by the folic acid antagonist aminopterin, which also counteracted the estrogen-induced increase in uptake of P^{32} into nucleic acid and phospholipide fractions (373). Hormone-dependent changes in the distribution of phosphoric acid esters in the rat uterus in relation to the age of the animal were also studied (369, 370, 371).

The presence of proteases (374) and of 6- β -hydroxylase (375) in human placenta is reported. Other interesting substances detected in this organ include ferritin, present in the cytoplasm, but not the nuclei, of placental cells (376, 377). The high blood content of human placenta at term (378) calls for caution in the interpretation of biochemical findings. Placental tissue (guinea pig) contains vitamin B₁₂ (379), and is relatively rich in methionine (380). Data have been obtained on the nucleic acid content of cellular fractions prepared from human placenta (381). The isolation and separate estimation of estradiol-17 β , estrone and estriol was accomplished (382) following upon earlier reports on the occurrence in the placenta of free and protein-bound estrogen. The values showed a not unexpected range of variation: (in $\mu\text{g./Kg.}$ placental tissue) estrone 8.5–111.5, estradiol-17 β 52–315, estriol 50–1030.

Placental permeability represents an ever widening field of study; problems, the solution to which is eagerly sought, include the pathway whereby passive immunity is transmitted from mother to fetus in advanced pregnancy (383, 384); selectivity of the yolk sac splachnopleur (385); the transport of antibodies from yolk sac to the circulation of the embryo (386). The passage of various more or less complex chemical compounds from mother to embryo was investigated in primates, and in domestic and laboratory animals; among the substances tested were chlortetracycline (387); D- and L-histidine (388); sugars such as glucose, fructose, xylose (201, 389, to 392); azo-dyes (393). Studies of considerable physiological and ultimately also clinical interest were continued on the role of the placenta in regulating the exchange of iodine and thyroid hormones between mother and fetus (394, 395, 396); there is evidence that the placenta takes up iodine readily, especially where it is administered to the mother; the thyroid hormone is also concentrated by the placenta; the uterus retains iodine markedly but unlike ovaries, does not fix the hormone. The maternal-fetal relationship was also studied as regards goitre formation in the fetus; when perchlorate was given to guinea pigs in the third week of pregnancy, fetal but not maternal goitre was produced (397), large doses of triiodothyronine failing to protect the fetus from perchlorate goitre: presumably perchlorate ions crossed the placenta and evoked a goitre response owing to the sensitivity of the intrinsically hyperplastic fetal thyroid to iodine depletion, the ineffectiveness of triiodothyronine being due to the placenta barring its prompt entry into the fetus, and to subsequent destruction of the hormone. In women at term significant amounts of thyroxine or triiodothyronine were capable of passing the placenta but at a slow rate; perhaps this explains the relative inadequacy of maternal hormone supply where there is some primary thyroidal defect in the embryo (398).

Pregnancy.—Endocrine relationships in pregnancy and the interdependence of the maternal and foetal endocrine systems have received much attention as evident from studies on the manifold aspects of adrenal (399 to 402) and thyroid (403) influence upon reproductive performance; the effect of ovarian hormones and related steroids and of relaxin (404, 405, 406) on the

maintenance of pregnancy, parturition, and rearing of litters; the effect of growth hormone on the length of gestation (407). The peculiar effect which lactation exerts on the implanting blastocysts remains as intriguing as ever in spite of continued efforts to elucidate the endocrine conditions responsible for the delay in implantation (408, 409, 410).

Perhaps the most fascinating account of the year concerns adaptive viviparity and the mechanism of gestation in a little exotic toad *Nectophrynoides occidentalis* (411, 412, 413). This but recently discovered 22 mm. long creature breeds in an arid mountainous environment which virtually excludes the tadpole phase; it has a 9-months' gestation period and produces fully metamorphosed 9 mm. young. In the actual parturition the uterus does not respond to oxytocic stimulation, instead, two factors intervene, namely the inflation of the lung sacs coupled with thoracic region (muscular) contractions; in addition, the parturient animal requires the mechanical support of suitably formed terrain to make delivery possible. This latter factor was not at first appreciated and caused total reproductive failure in captivity.

Contributions have been made representing notable advances in chemical embryology; the presence was shown of a potent growth-stimulating factor, namely vitamin B₁₂, in the unimplanted mammalian embryo as well as in its immediate environment, that is the uterine secretion (414), of the nature of which regrettably little is otherwise known. There are new data on the composition and formation of mammalian foetal fluids and membranes (415, 416, 417, 418); remarkable changes are reported, in the content of nucleic acid and nucleotides, and in several vitamins of the B-group, during the development of invertebrate embryos (419, 420).

Nothing illuminates more clearly the vulnerability of early embryos (mammalian, avian) than the numerous reports on a wide variety of extraneous agents capable of interfering with normal foetal development, the intensity of the damage ranging from complete cessation of growth to characteristic lesions and impairment resulting in malformations. In this category belong the antimetabolites 5-oxo-L-nor-leucine (421) and 6-chlorpurine (422); certain antioxidants (423); semicarbazide HCl (424); viruses (425); serum of cancer patients (426). Conditions which also bring about deleterious fetal changes include limited periods of pteroylglutamic acid deficiency (427); fluctuations in environmental temperature (428, 429); amniotic sac puncture for sexing human embryos (430); administration of sex steroids especially estrogens (431); maternal immunization against organ tissues (432).

The mechanism of implantation, that crucial stage in embryonic development, was discussed in relation to the rat (433, 434), armadillo (435), grey seal (436); in more advanced fetuses studies were done on the rate of growth of the reproductive tract (437); the innervation of the foetal genitalia (438) foetal circulation (439, 440, 441, 442); alkaline glycerophosphatase distribution in limb buds (443). An interpretation is given of reproductive anomalies (444) and of events in the foetus which determine sex differentiation and the development of accessory organs (445).

The presence is reported of 5-hydroxytryptamine in human pregnancy

and post-partum urine (446) and of an antidiuretic substance not identical with 5-hydroxytryptamine, in pregnancy serum (447). There is evidence that the mechanism of urea formation in pregnant rats is partially influenced by the placenta which withdraws, and thereby shifts, amino acids from the maternal into foetal circulation (448).

A critical evaluation of biological pregnancy tests is available (449). Surveys of practical significance give information on pig breeding records (450) and the relation between pregnancy and the occurrence of estrus in pigs (451).

NUTRITION IN RELATION TO REPRODUCTION

The influence of reduced food intake on reproduction was studied in cattle (164, 452), sheep (453, 454), and dogs (455, 456). In bull-calves, restriction of food intake had a much stronger delaying effect on the onset of androgenic activity than on the spermatogenic function (164). New evidence was presented on the importance of dietary calcium, phosphorus, and manganese for the reproductive efficiency of cattle (457, 458) and on the relation of manganese to normal estrus in pigs (459). Massive doses of iron injected to rats in the form of an iron-dextran complex produced degenerative changes in the testes, similar to those which occur in vitamin E-deficiency (460).

Further studies have been reported on the effect of maternal hypovitaminosis A upon the incidence of hydrocephalus in the young rabbit (461) and upon the occurrence of reproductive disorders in cattle (462) and rats (463). Hypervitaminosis A was observed to produce teratogenic effects in rats (464, 465); the administration of vitamins A, B, C, D, and E to rats exposed to a single dose of x-radiation early in pregnancy increased markedly the incidence of anophthalmia in the foetuses (466). The need for an adequate supply of vitamins of the B-group was again shown to be of paramount significance in maintaining the fertility and foetal development of rats (467), and more experimental work was done on the role of dietary protein in the normal development of sexual functions in growing rats (468).

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PHARMACOLOGICAL ASPECTS OF PERIPHERAL CIRCULATION^{1,2}

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The continuous progress in pharmacological research of recent years has led to the identification of both natural and synthetic substances which when added to the list of classical drugs constitute a series of compounds possessing biological activity and mechanisms of action unknown a few years ago. Before analyzing the pharmacological action on the various circulatory regions it is necessary to put forward a classification which, although not rigid, may correspond to the actual state of our knowledge concerning drugs possessing vascular activity. Following the concept previously described (52), all those chemical compounds which act in a direct or indirect way on the same receptors will be listed together with the natural products possessing vascular activity (epinephrine, acetylcholine, histamine, 5-hydroxytryptamine). Then the groups of drugs which influence the peripheral circulation indirectly by acting on the nervous control of the circulation will be considered. Finally an indication will be given of compounds with a polypeptide structure which, although little is yet known about their mechanism of action, represent a group of substances which year by year are assuming an ever increasing importance in the pharmacological and physiological field.

THE PHARMACOLOGY OF CHEMICAL MEDIATORS AND OF RELATED COMPOUNDS

For each of the major groups of mediators, Table I gives the biological derivatives, the mimetics, precursors, competitive antagonists, liberators, inhibitors and sensitizing agents.

The epinephrine group.—To the classical sympathetic mediators, epinephrine and norepinephrine (119), isopropyl-norepinephrine has now been added. In fact sympathetic stimulation of the cat heart-lung preparation causes the appearance in the blood of the pulmonary veins of a sympathin of bronchial origin which from its pharmacological and chromatographic characteristics corresponds to isopropyl-norepinephrine (248). At present it is not possible to specify whether this phenomenon is limited to the bronch sympathetic nerve endings or whether it may extend to other areas. The sympathetic vasodilator nerves of the muscles seem to act by an acetylcholine-type of mechanism (84, 129). By contrast the nerve fibers, which are responsible for the cutaneous vasodilation on application of heat, are not certainly cholinergic in nature (329). Mohme-Lundholm (276) maintains that, both for epinephrine and isopropyl-norepinephrine, the relaxing

¹ The survey of literature pertaining to this review was completed in May, 1957.

² The authors are indebted to Dr. John Daymond for preparation of the text in English.

TABLE I
COMPOUNDS RELATED TO THE CHEMICAL MEDIATORS

	Epinephrine and Norepinephrine	5-Hydroxytryptamine	Histamine	Acetylcholine
Biological Derivatives	Isopropyl norepinephrine	Bufofentine (114)		Propionylcholine Murexine (118)
Mimetics	Sympathomimetics Phenylephrine Epinephrine Amphetamines	5-Methoxytryptamine Tryptamine (116)	α -Pyridylethylamine	Parasympathomimetics Methacholine Carbachol Bethanechol
Precursors (a) Physiological precursors (b) Drugs acting after transformation in the organism	(a) Tyrosine DOPA (42)	(a-b) 5-Hydroxytryptophane	(a) Histidine (43)	(a) Choline Acetyl donors (b) Chloropyruvic acid
Competitive Antagonists	Sympatholytics Ergot alkaloids Prosympal Pentolamine Dibenzamine Antagonists of sympatholytics	LSD and its derivatives Aminoindoles Nitroindoles Medmain	Antihistamines Pyrimilamine Triphenylamine Chlorphenamine Promethazine	Parasympatholytics Atropine Ganglion blocking agents TEA, Pentamethonium Curare and curare-like drugs "Ganglioplegiques centraux" (250)
Liberators	Reserpine Morphine (191, 357)	Reserpine Amphetamine 48/80	Histamine liberators 48/80, egg white, dextran, Tween 80	Potassium ions
Inhibitors of (a) Liberation (b) Synthesis	(a) Iproniazid (b) 2:6 xylyl-ether bromide	(a) Iproniazid	(a) ATP, metabolic inhibitors, HSC, quaternary ammonium bases, Azulene ?	(b) Choline acetylase inhibitors (30), botulinus toxin (?), antimalarial drugs, HC3 (252)
Inhibitors of Enzymatic Destruction	Monooxygenase inhibitors Iproniazid	Monooxygenase inhibitors Iproniazid	Diaminoxidase inhibitors Aminoguanidine	Anticholinesterases Physostigmine, Prostigmin, organic phosphate esters Specific antagonists of acetylcholinesterase and serumcholinesterase Reactivators of phosphorylated cholinesterase, PAM, DAM
Sensitizing Agents (mechanism of action dubious)	Cocaine, local anesthetics Phenol derivatives with antioxidant properties		Pertussis vaccine (255)	

References are only given of those substances which are not further discussed in the text.

action on smooth muscle depends on an increased liberation of lactic acid caused by the amine. This concept contrasts sharply with the persistent relaxing action observed *in vivo* on the isolated intestine with low glycogen content in the presence of substrates which supply nonglycolyzable energy (28, 139).

With regard to the large group of the sympathomimetic drugs, recent research has been mainly directed to the study of compounds of the phenylisopropylamine type possessing slight peripheral vascular and high central stimulating activity. Their effects on the cardiovascular system, like those of ephedrine but unlike those of the true sympathomimetic drugs, may be blocked by cocaine. Atropine is also able to abolish the hypertensive effects of these drugs, by means of some unknown mechanism (164). Compounds with an exclusively peripheral vasodilator action have been obtained from sympathomimetic amines by substituting in the amino group a long aliphatic or arylaliphatic chain (64). The same type of substitution on the amino group of aliphatic sympathomimetic compounds produces analogous vascular effects (163). Apart from the series of phenylethylamines, an imidazole derivative of 1,2,3,4-tetrahydronaphthalene (tetralene) has been described with a stimulating and inhibiting peripheral vascular activity which is similar to that of epinephrine but with a persistent hypotensive action (123).

In the field of the adrenolytics a large number of the new compounds described belong to the groups already noted (53, 290). However, the most interesting point is the existence, in numerous substances of this group, of central actions of a general sedative nature. These effects are widely acknowledged for the phenothiazine derivatives (225, 259, 291, 292, 295, 349) and have already long been described for the hydrogenated ergot alkaloids (219). They are also found in a derivative of benzodioxane (141), in an aminoketone which structurally represents an intermediate phase between the benzodioxanes and the analogous derivatives of tetralene (322), in a semisynthetic alkaloid from yohimbine (270), in Raubasine, an adrenolytic alkaloid from *Rauwolfia* (348), and in some derivatives of piperazine (275, 376). The definite central sedative effects superimposed on the adrenolytic action account for the difference in the hypotensive action of the compounds. From a theoretical point of view a good demonstration of the competitive antagonism between adrenolytics and sympathetic chemical mediators has been provided by the observation that *N*-(2-chloroethyl) dibenzylamine hydrochloride (Dibenamine) increases the quantity of norepinephrine released in the venous blood of the spleen after sympathetic stimulation (61). Finally Hermann (179), in his experiments on dogs with ablated spinal cords, demonstrates the competitive nature of peripheral adrenolytic action, not only with regard to the vasoconstrictor effects of epinephrine but also to the vasodilator action of the sympathetic hormone which is inhibited by vasodilator sympathomimetic derivatives. An antagonism to the inhibitory action of the sympathomimetic amines has also been obtained with ephedrine (241) and with a chloro analogue of isuprel (362).

A new group of substances is represented by those compounds which liberate norepinephrine from sympathetic nerve endings, from the central nervous system, and from the suprarenal medulla. An example is reserpine which produces a progressive and lasting disappearance of cerebral, myocardial, and ganglionic norepinephrine and of suprarenal epinephrine and norepinephrine (34, 191, 288, 358). The liberation of the sympathins depends on a direct cellular or subcellular action. Only in the case of the suprarenals is a part of the effect of reserpine attributable to a sympathetic stimulation transmitted to the glands by the splanchnic nerves (69, 191, 228, 381). The action of reserpine on the cerebral norepinephrine may be blocked by isopropylisocotinyldiazine (iproniazid), as has already been observed in the case of 5-hydroxytryptamine (69, 357). Eade (111) has described the release of epinephrine by the action of histamine liberators on the submicroscopic granules which appear to constitute the intracellular deposit form of the mediators (44, 45, 120). Two types of pharmacological effects are derived from this action of reserpine. On the one hand the peripheral effects of the liberated mediators including hypertension in the animal treated with ganglioplegics, retraction of the denervated nictitating membrane, (263) and hyperglycaemia (380), and on the other hand the inability of the sympathetic nerves deprived of norepinephrine to respond to electrical or chemical stimulation (34, 69).

The existence of a new type of compound capable of interfering with the sympathetic nervous activity might perhaps be suggested by the observation of Exley (121) that 2,6-xylylcholine ether bromide (TM 10) is able to inactivate the sympathetic nerve endings by blocking the synthesis of norepinephrine (17). It is difficult to say whether this action is responsible for particular pharmacological effects owing to the wide variety of effects exerted by the drug (405). However, TM 10 has already aided the solution of several theoretical problems concerning the origin of isopropyl norepinephrine from the bronchial sympathetic nerves (248).

Among the substances capable of interfering with the metabolism of the sympathetic mediators, iproniazid is the most active drug inhibiting monoaminoxidase. Without transgressing the limits of the present discussion it may be observed that it is still uncertain how important the enzyme may be in the inactivation and perhaps also the control of the synthesis of the sympathetic mediators (21, 42). It is, however, certain that: (a) the inhibition of monoaminoxidase by iproniazid does not alter the peripheral response to catecholamine, although it may increase the action of some non-phenolic sympathomimetic amines (21, 81, 347); (b) a possible potentiation of epinephrine or norepinephrine by iproniazid seems to depend on non-specific phenomena (203); and (c) the blocking of the monoaminoxidase by iproniazid does not increase the quantity of norepinephrine which appears in the venous blood of the spleen on stimulation of the sympathetic nerves (61).

Excluding the possibility that a potentiation of the sympathetic media-

tors may derive from blocking of the aminoxidase it is suggested that the sensitizing substances (such as cocaine or ephedrine) inhibit a hypothetical enzyme which attacks the sympathomimetic amines at the metahydroxy group position (139). Other authors (21, 394), however, favor the concept of pharmacological denervation (126). According to this concept the substances (including cocaine) capable of blocking nervous transmission abolish the continuous liberation of mediators at the sympathetic nerve endings, consequently cancelling a supposed state of adaptation to norepinephrine by the effector cells. This interpretation might also be applicable to the potentiation produced by different substances. Thus the sensitization to epinephrine produced by ganglioplegics, which is observed after transection but not after removal of the spinal cord (24), may depend on a pharmacological denervation. The potentiation of epinephrine by reserpine (8, 172) may even result from functional sympathetic inactivation produced by the disappearance of norepinephrine from the sympathetic nerve endings. For a series of piperazine derivatives possessing epinephrine potentiating activity an action on permeability has been proposed (231) in accordance with the classical theory of Cannon & Rosenblueth (333). With regard to membrane phenomena it is not improbable that variations in the ionic equilibrium may also modify the vascular activity of the sympathetic mediators. It has been shown that the vessels of animals with an abnormally high sodium chloride content show a hypersensitivity to epinephrine *in vitro* (395). A more extensive study in this direction would also be useful in relation to problems of hypertension in man and of the various forms of experimental hypertension. As regards ionic factors a decreased reactivity towards epinephrine has been observed in calcium (387) or potassium (136) depletion. Even more obscure is the influence on the vascular activity exercised by the endocrine glands (57, 298), the kidneys (390), bacterial endotoxins (382, 413), iron salts (86, 379), cyanide poisoning (180), and carbonic acid (381).

The acetylcholine group.—Although the significance of acetylcholine as a local hormone has been confirmed (65, 145, 368), the presence of propionylcholine in mammalian spleen extract is discussed (178).

Some new observations concern the pharmacological activity of a purified preparation of muscarine, a new structural formula which has been described by Kögl *et al.* (83, 215). Together with the typical peripheral actions (134, 229) nicotinic effects on the perfused superior cervical ganglion have also been described (8, 220). This latter action is not surprising if it is considered that atropine has ganglioplegic effects on this preparation (218).

The study of the different aspects of action of acetylcholine is essentially of a pharmacological nature. Various suggestions have been advanced on the amphoterism of the receptors responsible for the nicotinic action (297), the significance of the cationic and anionic portions of the acetylcholine molecule with regard to its muscarinic and nicotinic action (317), and the effect of substitution of these two fundamental parts of the molecule on its cholinergic and lytic activity (175, 233, 352, 383, 393). Among the choline-

mimetics the muscarinic action of halogen derivatives of pyruvic acid is attributed to their rapid transformation in the organism into choline esters (67, 260), while atropine-like substances have been obtained by the preparation of quaternary compounds of antihistamines (280) and spasmolytics (190). In the field of the alkaloids of the tropane group, the different types of stereoisomers, and the relationships between the structure and activity of these substances, have been investigated (162, 183).

From a hemodynamic point of view the most interesting compounds are still those which antagonize the nicotinic action of acetylcholine or ganglioplegics. Together with the classical compounds consisting of two quaternary ammonium groups symmetrically joined together by polymethylene chains or more complex radicals, ganglioplegics have been described possessing two asymmetrical ammonium groups (4, 122, 214, 247), two symmetrical sulphone groups (23), and finally with a single tertiary amino nitrogen attached to the large molecule of isocamphane (378). With this compound, in addition to the hemodynamic effects characteristic of ganglioplegics (106, 131, 364), central actions have been described in the experimental animal (377) and in man (350) which are attributed to an easy penetration of the drug into the central nervous system. These more recent types of ganglioplegics would suggest that only one of the positive groups is destined to attach itself to the ganglionic receptors, while the remainder of the molecule serves as a barrier between the receptors themselves and the mediators of ganglionic transmission (122), analogous to that which atropine-like substances are believed to offer (233). In this way the structural differences between the atropine-like substances and the ganglioplegics are minimized. It is thus possible to establish a link between the products of the first and those of the second type by means of compounds possessing intermediate structural characteristics and activity. Thus, the quaternary derivative of 6-acetoxymethoxyethyl-dimethylamine (opilon) has atropinic and slight ganglioplegic properties (190); butylscopolammonium has stronger ganglioplegic atropine-like activity; and mecamlamine, which is structurally not very different from butylscopolammonium yet has pure ganglioplegic action analogous to that of the classical compounds. On the basis of these considerations it is easy to understand why certain ganglioplegics have an atropinelike action which reinforces the effects of the ganglionic block only in the parasympathetic section of the autonomous nervous system (190, 226). Nevertheless even within the limits of the pure ganglioplegics the possibility of a prevalently parasympatholytic action has been confirmed (316) as has the existence of ganglionic synapses which are more resistant to this blocking action (371). As regards the mechanism of hypotension produced by ganglioplegics, good agreement has been observed between the mydriatic and hypotensive activity in a wide series of products (41). On the basis of clinical and experimental research the hypotension induced by ganglioplegics appears to result not so much from a fall in the peripheral resistance as from a peripheral "pooling" of blood which produces a reduction in the cardiac output (88,

262, 367). This would agree with the well known clinical phenomenon of pronounced orthostatic hypotension observed during the course of treatment with ganglioplegics (367).

The theoretical possibility of the existence of acetylcholine-liberators with an action equivalent to that of the histamine or epinephrine-liberators has been suggested by Loewi in discussing the intraneural state of acetylcholine (249). In this connection it would be interesting to study the liberation of acetylcholine by the potassium ion (257), especially in relation to the new observations concerning the association of ionic displacement with stimulation phenomena (125) and the inhibition of the physiological liberation of acetylcholine by botulinus toxin (313), an action which is antagonized *in vitro* and *in vivo* by succinodinitrile (148, 149).

The biosynthesis of acetylcholine has been widely reviewed (258), while from a biochemical viewpoint the sulphhydrylic nature of cholinacetylase has been established (30). Same antimalarial products (51) and a hemiacetal derivative containing two molecules of choline (252) have been shown to inhibit the synthesis of the neurohormones. Data is lacking on the possible hemodynamic action of these compounds but it is interesting to note that a flavonoid which inhibits the synthesis of acetylcholine is able to reduce the toxicity of anticholinergic drugs (20).

In the group of anticholinesterases new nonaromatic (14) and aromatic (217) carbamates and also compounds obtained by doubling the neostigmine molecule have been made available (182). In a series of biquaternary products the effects of introducing phenolic and carbamic groups on the specificity and irreversibility of the anticholinesterase action have been studied (199). The vascular effects of these substances correspond to the various levels of the nervous system on which they exert their action. Thus in the rat, arterial hypertension is observed with physostigmine and tetraethylpyrophosphate which reach the vasomotor centers, while hypotension results with neostigmine which cannot overcome the blood-brain barrier (194). In the unanesthetized rat the vascular responses to physostigmine are variable, sometimes a change from hypertension to hypotension is observed (268).

An accurate analysis of the peripheral vascular reactions of the dog to tetraethylpyrophosphate has revealed intense muscular and cutaneous vasoconstriction which depends on a number of factors, such as anoxia, stimulation of the vasomotor centers, increase of ganglionic transmission, and stimulation of the chemoreceptors (97). Finally, there is the group of substances related to these drugs which inhibit the action of the anticholinesterases. Apart from some plasma enzymes capable of inactivating organic phosphoric compounds (5), numerous derivatives of hydroxamic acid may reactivate cholinesterase blocked by organic phosphate esters (207, to 210). The reactivation is still limited since the compound resulting from the reaction between the enzyme and the phosphoric acid is only labile in the first phase of the reaction, becoming transformed later into a stable phosphoenzymatic derivative (187). One may thus explain the strong pro-

phylactic but weak therapeutic action of hydroxamic acid in organic phosphate ester poisoning (12, 251, 406). Anticholinesterase inhibitors may be effective against sublethal doses of organic phosphate esters (251, 312), especially if associated with atropine (62, 406).

The histamine group.—The physiological significance of this substance, present in such large quantities in the organism, is still a widely discussed but as yet unsolved problem. Of the numerous contributions it will be useful to refer to the views of various authors expressed at a recent symposium and in particular to the conclusions of Whelan (404) on the hypothetical intervention of histamine in the phenomena of physiological vasodilation. The role of histamine in anaphylactic phenomena is better known from the combined clinical and experimental observations of Lecomte (236). The liberation of histamine by *in vitro* contact with the antigens from parts of the organs of sensitized animals has lately been confirmed and it is established that the presence of calcium ions in the medium is essential for histamine liberation; the same phenomenon is also verified on the subcellular granules (278).

In the large group of antihistamines, of which Protiva has collected and analyzed more than 5000 compounds (324), an interesting series is that containing a thionaphthindolic nucleus and possessing an antihistaminic activity of slow appearance and long duration (321); in spite of the structural resemblance to the phenothiazines these derivatives seem practically devoid of central activity. In these products the introduction of a halogen in certain positions distinctly reinforces the antihistaminic activity. This property is also found in other pharmacologically active molecules as has been observed by Cavallini *et al.* with respect to derivatives of benzyimidazoline with sympathomimetic and lytic activity (75). The vascular actions of the antihistaminics are few and of a nonspecific nature, and this represents one of the main obstacles to the hypothesis that histamine may have a physiological role in the control of the circulation. On the other hand the possibility should not be excluded that histamine may pass from an inactive to an active form in the effector cells (404) where the antagonists are unable to penetrate. In this case the phenomenon would be analogous to that commonly observed for the adrenolytics, which are much more active in inhibiting the action of the epinephrine circulating in the blood than in opposing the effects of sympathetic stimulation, as has recently been confirmed (345, 346). In the case of the antihistaminics the difference between the inhibition of the circulating histamine and of the endogenous histamine would thus be carried to its extreme limit.

The study of products with histamine-liberating activity, which was initiated a few years ago, has already given rise to a large number of substances which constitute a fairly heterogeneous group (310). To these have now been added some active principles extracted from intestinal parasites (189), vegetable parasites (388), medusa (189), and the mammalian kidney (384). These substances cause lysis of the mastcells with subsequent libera-

tion of the biological amines contained within them (281). This explains why in dogs with mastcell tumors the hypotensive action of 48/60 is much more intense than in normal dogs (234). If the more apparent hemodynamic effects of histamine liberators are attributable to the liberation of histamine (167), other actions seem to depend on the liberation of heparin (40) and 5-hydroxytryptamine (7, 37, 168, 223, 306, 308, 337) which are also present in the mastcells. The toxic action is only partly due to the liberation of histamine (282). In this connection it should be noted that the liberation of histamine and of 5-hydroxytryptamine does not seem to be the same for all the products of this group (308). The problem of the mechanism of action of these compounds is connected with that of the inhibitors of the pharmacological and anaphylactic liberation of histamine, amongst which are described ATP (92), metabolic inhibitors (277), some quaternary ammonium bases (266), some vegetable extracts rich in pectins [HSC (188)], azulene (375) and some hormone factors which have not been clearly defined (150). The often contradictory results obtained and wide variety of methods employed in this field for the liberation of histamine make an assessment difficult. It is only certain that notable differences exist, not only between the anaphylactic and pharmacological liberation of histamine, but also among the various types of chemical liberators, as may be deduced from the observation that the appearance of tachyphylaxis to a specific histamine liberator does not prevent other compounds of the same group from being fully active (222, 363).

In the field of substances which interfere with the metabolism of histamine, diverse inhibitors of diaminoxidase (aminoguanidine, β_1 -pyrimidine, stilbamidine, isonicotinylhydrazine) have displayed activity by specifically increasing the vascular effects of histamine when injected into the renal artery (244). The kidney is the principal organ which inactivates histamine because it is rich in diaminoxidase (243). It should be noted that the lack of potentiation of histamine injected intravenously does not support the view that the vascular action of this substance is normally limited by its destruction by diaminoxidase. Furthermore, the effects on the urinary excretion of histamine seem to be rather small. In man a slight increase in the elimination of histamine injected subcutaneously is observed (274), while in rats, treated with histamine liberators, aminoguanidine does not produce any increase in elimination of histamine (239), histamine toxicity is not increased by the majority of diaminoxidase inhibitors (10).

As has already been stated for epinephrine, the study of the influence of variation in the ionic equilibrium on the vascular activity of histamine is still in the initial stages. It is known that an increase of the K^+ concentration produces an increase of the activity to histamine of isolated vessels *in vitro* (366).

The 5-hydroxytryptamine group.—Although 5-hydroxytryptamine (5-HT, serotonin) presents enteramine structural and pharmacological analogies with the sympathomimetic amines on the one hand and with histamine on the other, it is preferable to consider it independently in order to analyze the

numerous problems raised by the discovery of this biological amine (114, 301). The vascular activities of 5-HT have been the first to be studied. At the present time, it is possible to distinguish two mechanisms of action on the peripheral circulation (351). The first is represented by the direct vascular effects which resemble in some respect those of both epinephrine, in the type of action (vasoconstriction and vasodilation) and in the behavior towards specific inhibitors (46, 341). The second mechanism includes all the indirect actions of 5-HT, from those well known to act on the Bezold-Jarisch reflex, which will be dealt with later, to the more hypothetical ones depending on a supposed central sympathetic and direct suprarenal medulla activation (39, 400); or alternatively associated with depression of neurogenic tone which opinion is still maintained (302). The vascular effects of 5-HT are undoubtedly different depending on the species of animal and also the method of administration. The rapidity with which the platelets fix injected 5-HT is a factor to which different authors have drawn attention (1, 254), but requires further explanation in the interpretation of the effects of the slow infusions of 5-HT. So far, only the variations of the 5-HT content of the platelets after rapid intravenous injections have been studied (56). With regard to the vascular action it may be concluded that the pressor activity of 5-HT has little physiopathological importance. One point alone would seem to be sufficient to validate this fact, namely, the absence of significant alterations in the arterial pressure in clinical forms of carcinoid, that is a tumor formed by the cells producing 5-HT (143). The clinical and pathological study of patients with these tumors has revealed a possible connection between 5-HT and edemata and connective proliferation (11, 253, 267) which may be related to the experimental observations on the importance of 5-HT as a factor in edema from ovalbumine, from dextran, and from 48/80 (7, 204, 223, 306, 307, 308, 337). If the existence of these histamine-like actions is proved, it would suggest that 5-HT, like histamine, acts as a chemical intermediary of anaphylactic reactions (124, 304, 398, 401).

Although little is known about 5-HT-like substances more is known of the group of the antagonists. In view of the multiple epinephrine- and histamine-like activities of 5-HT, it is logical that numerous adrenolytics are capable of antagonizing the vascular activity of 5-HT (269) and that some antihistaminics inhibit the bronchoconstriction caused by 5-HT (211, 269). The antimetabolite concept of Woolley *et al.* (355, 408, 409) suggests the possibility of obtaining substances specifically antagonistic to 5-HT by modifying the fundamental structure of its molecule. The hypotensive activity which some of these derivatives exert in clinical hypertension suggested that 5-HT plays a role in the pathogenesis of this condition (409). However, this interpretation finds no further support from clinical and experimental data. Among the specific antagonists, the diethylamide of lysergic acid (LSD) is certainly the most noteworthy. Many of its derivatives are capable of inhibiting biological actions of 5-HT (76, 77) and of these, the most studied is the bromo derivative which seems to be less specific than LSD,

and also possesses a slight adrenolytic action (338, 339). The activity of these drugs is different in the various animal species. In the rat, LSD and its bromo derivative are very active in inhibiting the vascular action of 5-HT, in the cat they are less active, while in the dog they are practically without effect (338). The vascular effects of LSD and the bromo derivative are inconspicuous the latter having a slight hypotensive action, the former having an irregular hypertensive effect. This activity probably results from either direct vascular effects or central stimulation. This is accompanied by various manifestations in experimental animals, such as hyperglycaemia (216), hyperthermia (146), and constitutes the most interesting aspect of this compound from the point of attempting to link the hallucinogenic action of LSD in man with its inhibiting action on 5-HT. This problem only indirectly concerns the present discussion, but the main facts which contradict this hypothesis are here reported. (a) Many derivatives of LSD which antagonize 5-HT are not hallucinogenic (334) and have little central stimulatory action in the experimental animal (216); (b) LSD exerts its central action even after the administration of reserpine (146, 198), that is after a large part of the cerebral 5-HT has been liberated; (c) the central actions of LSD persist even after disappearance of this substance from the brain (166). This last fact is also in contrast to the possibility of a 5-HT-like action of LSD at the cerebral level, as suggested by some authors (48, 402) on the basis of the conception that a central stimulatory effect is attributed to 5-HT and its precursor, 5-hydroxytryptophane (47, 356).

One of the pharmacological actions of major interest is that of substances liberating 5-HT. In 1955 Brodie *et al.* (59) described the disappearance of cerebral 5-HT in animals treated with reserpine, and this action recently has been confirmed not only for the brain but also for many tissues containing 5-HT: the intestinal mucosa (33, 82, 115), the platelets (115, 154, 289), the spleen (33, 115), and the mastcells (38). The liberation of 5-HT produces an increase in the urinary elimination of 5-hydroxyindolacetic acid (116, 135). Clear differences exist, however, in the different organs; thus the intestinal 5-HT is less sensitive to reserpine than that of the brain (82). The mechanism of this action is unknown; in this case iproniazid also inhibits the tissue depletion of 5-HT (35, 320, 357) but it is difficult to state whether this is due to an inhibition of 5-HT liberation or an increase in the synthesis and reduction in the destruction of 5-HT. The observation that iproniazid increases the cerebral content of 5-HT (320) would appear to favor the second type of action. The biological significance of the liberation of 5-HT is still an unsolved problem as it is not yet known what role this substance plays at the central level. Some investigators have suggested that the hypotensive action of reserpine results from the liberation of an active form of 5-HT (87) and others have proposed that this action depends on a cerebral depletion of 5-HT (100). It has been observed that iproniazid, although inhibiting the central sedative action of reserpine, does not significantly alter the hypotensive effect (35). Finally, it should be recorded that

amphetamine, a substance possessing typical central stimulating activity, also gives rise to cerebral liberation of 5-HT (300); a similar action of reserpine and of iproniazid is also observed with respect to cerebral nor-epinephrine. Thus any theory in this field would still appear to be somewhat premature.

Among the substances capable of interfering with the metabolism of 5-HT, iproniazid in addition to the effects already referred to, increases the vascular action of 5-HT (21) and decreases the urinary excretion of 5-hydroxyindolacetic acid which is derived from the oxidative deamination of 5-HT by means of monoaminoxidase (81). Thus aminoxidase appears to fulfill an important role in the biological inactivation of the amine (42).

DRUGS ACTING ON THE NERVOUS REGULATION OF THE CIRCULATION

Schematically the nervous control of the circulation may be considered as a reflex arc, the drugs of this group acting on the afferent, central, and efferent parts. The compounds with nicotine-like or ganglioplegic activity which exert their action on the efferent portion of the reflex arc have already been discussed. The drugs acting on the afferent part and the centers will be examined. Among the former may be considered the substances which influence the baroreceptors of the carotid sinus and aorta, the chemoreceptors of the carotid and aortic glomus, and finally the peripheral receptors of which the most important are those giving rise to the Bezold-Jarisch effect.

With regard to the baroreceptors of the carotid sinus recent work has confirmed that variations in tension of the wall of the carotid sinus represent an effective stimulus for the nerve endings contained within (336). The influence which different substances with myotropic activity may exert on the carotid sinus *in vitro* has been demonstrated (185). These local actions of the vasodilator and vasoconstrictor drugs not only affect the systemic circulation, but also the pulmonary circulation (391). Sympathetic denervation of the carotid sinus increases its sensitivity to locally applied sympathomimetic amines, but does not produce reflex modifications of the arterial pressure (264). Ether and chloroform are able to sensitize the baroreceptors thus producing marked arterial hypotension; this may explain the circulatory collapse sometimes observed with these anesthetics (328). It is well known that many substances with ganglion-stimulating or inhibiting action also exert qualitatively analogous actions on the chemoreceptors of the carotid glomus (184, 218, 232). The existence of a synaptic mechanism of transmission of the normal physiological stimulus of the cells of the glomus has recently been discussed (71, 105, 343). In general the drugs with ganglioplegic activity, or those potentiating ganglionic transmission, have an action qualitatively equal but quantitatively different on physiological and pharmacological stimuli. This question thus requires further investigation.

Although the baro- and chemoreceptors of the carotid sinus and the aorta have well defined functions and known effects on the circulation, the physiological significance and the circulatory effects of other receptors still

remain very obscure. From a pharmacological point of view the most studied are the cardiac and pulmonary-receptors, which on stimulation give rise to the appearance of the well known Bezold-Jarisch effect, characterized by bradycardia, hypotension and apnea (201). Numerous pharmacological agents, from potassium ions to the veratrine alkaloids, are able to provoke these effects (93). Kramer has endeavored to determine the pharmacological character of products possessing this action (224). To this group may be added some of the recently studied benzofurane derivatives (344). The clinical significance of these pharmacological actions is still not clear. They certainly play a significant part in the vascular effects of the veratrine alkaloids (332), the hypotensive action of which is not entirely due to cardiac effects (331); it cannot be explained by the central action (410) which is of the stimulatory type (68, 138).

Among the drugs which act on the arterial pressure by means of a central mechanism the most significant are the phenothiazine derivatives and the *Rauwolfia* alkaloids. In the phenothiazine group the existence of a hypotensive action of central origin is still the object of discussion because these substances are frequently also active adrenolytics (78, 221). However, many considerations support the concept that the vascular effects are of central origin (63, 372). To these have now been added some of the new piperidine derivatives, which though practically free from peripheral sympatholytic action, are able to produce arterial hypotension and inhibition of the carotid sinus reflex produced by occlusion (294). Analogous results have been obtained with the sulfoxide derivative of chlorpromazine which is its most important metabolite (279). For the *Rauwolfia* alkaloids, particularly reserpine and rescinnamine, the hypotensive action is clearly of a central nature since peripheral adrenolytic effects are absent and since a clear inhibitory action is exerted both on the carotid sinus reflex evoked by carotid occlusion and on the hypertensive effects produced by electrical stimulation of the hypothalamus (6, 9, 36, 172, 227). From a theoretical point of view, the observation that reserpine produces a depletion of cerebral norepinephrine and 5-HT, and that chlorpromazine is an antagonist not only of the sympathetic mediator but also of 5-HT (26, 79, 161), has suggested that the two biological amines represent the common substrate of the central action of these drugs. It is not possible at present to state whether norepinephrine alone is involved, or acts jointly with 5-HT, in these depressant phenomena. In favor of the theory that an exclusively noradrenolytic mechanism operates is the fact that a typical stimulant such as amphetamine reduces the cerebral content of 5-HT (300), and also the observation that none of the specific inhibitors of 5-HT have central sedative effects.

THE GROUP OF THE POLYPEPTIDES

The recent use of new analytical and synthetic techniques has permitted the first steps to be taken into the field of the pharmacology of the polypeptides with the structural definition and synthesis of oxytocin and vaso-

pressin (110). In the absence of accurate chemical data concerning the majority of the polypeptides classification can only rest on biological criteria. The effects on the arterial pressure provide a good criterion for classification even if in reality it is probable that these actions are not always the most important. Thus, in the group of the polypeptides with hypertensive action, the antidiuretic posterior pituitary hormone has pressor effects which, although clinically used and present in synthetic compounds (265, 392), do not represent a significant activity from the physiological point of view. Only in pathological conditions, as observed in patients suffering from orthostatic hypotension, is ADH capable of producing significant vascular effects which probably depend on a state of vascular hypersensitivity (399).

Also for the renin, hypertensinogen, and hypertensin systems, the pressor effects are of secondary importance in comparison with the new biological effects which renin has been shown to possess. The observations on the relationship between the renin content of the kidney and the suprarenal cortical activity seem to indicate that renin may have an influence on the renal control of the electrolytic balance (157 to 160). These observations tend to focus the work on the physiopathology of experimental renal hypertension in a direction different from that considered so far. It is more in harmony with the repeated observations recently confirmed (107) of the minor importance of renin in the hypertensive action and of the intervention of extrarenal factors, particularly the suprarenal, in the chronic phase of experimental renal hypertension (128). In this field the existence of a peripheral sensitization to sympathetic mediators in experimental renal hypertension (335) and in human hypertension (155) may depend on changes in the ionic equilibrium (238), as has already been considered in the section concerning the substances potentiating epinephrine. The nature of the amino acids present in hypertensin has been investigated by various authors (113, 240, 314, 361), some of whom have suggested the existence of both an active and an inactive form of hypertensin (359, 360).

The biological significance of the polypeptides with hypertensive, oxytocic, and antidiuretic actions which are formed by the action of pepsin on plasma (90) is still not clear, while that of the polypeptides with hypertensive activity present in extracts of the arteries (101) and the submaxillary glands of white mice (403) is quite unknown.

With regard to the polypeptides with hypotensive action, it has been observed that nervous or pharmacological stimulation of the submaxillary gland of the cat provokes the appearance in the perfusate of an enzyme forming bradykinin. It is to the liberation of this polypeptide that one may attribute the vasodilation observed in the glands during functional activity (186). Bradykinin would thus assume physiological significance as a local regulator of the circulation. This hypothesis may possibly explain the incomplete inhibiting action of atropine on subcutaneous vasodilation produced by heat (329). The nature of the enzyme present in snake poison and responsible for the liberation of bradykinin has been studied from a strictly bio-

chemical point of view. It is apparently identical with an esterase of the methyl ester of benzoylarginine (169). Bradykinin liberator activity has been described in human plasma (242) and in bacterial extracts (323).

Less important from the point of view of the circulation, seems to be substance P, the action of which is reported mainly on the nervous system (85). Effects have been described of a central sedative (411, 412) and myanesin-like type (374) for substance P the new physiological role of which is discussed (386.)

Lately investigations in this field have led to the study of the pharmacological action of some synthetic polyamino acids. In the case of derivatives of glutamic acid, a capillary permeability action has been observed not attributable to liberation of histamine and susceptible to inhibition by cortisone (373).

REGIONAL VASCULAR REACTIONS PRODUCED BY DRUGS

The modifications produced by a drug in any particular vascular region depends not only on its characteristic local and systemic effects, but also on the state of the systemic circulation and the efficiency of its general and local autoregulation mechanisms. The efficacy with which these factors operate in the action of the drug varies according to the method of administration of the active substance (intravenous or intra-arterial) and the experimental conditions employed. In this part of the review an attempt will be made to demonstrate how these considerations may explain some of the apparent contradictions encountered in this field.

Coronary circulation.—The coronary circulation is strictly controlled by local factors which tend to guarantee an adequate oxygen supply for the energy requirements of the myocardium (165, 235, 271). These autoregulations on a metabolic basis are fundamental in the coronary reactions to the sympathetic mediators and sympathomimetic amines, the vasodilator actions of which are observed in the absence of general hypertensive effects (18) and appear to be strictly related to the chronotropic and positive inotropic action of these drugs (31, 102). Isuprel alone seems to produce coronary dilation at least partly directly (102), whereas epinephrine and norepinephrine, in the absence of myocardial reactions, tend to be vasoconstrictors (31, 213). Some experiments conducted on the whole animal have nevertheless shown an increase in the oxygen saturation of the coronary venous blood following intravenous injection of epinephrine or norepinephrine (245). In this case the increase of the coronary flow was greater than that necessary to compensate for the increased metabolic requirement of the myocardium. It is probable that this phenomenon depends on the systemic hypertensive action of the intravenously injected sympathetic mediators. However, it cannot be excluded that besides factors other than oxygen consumption may intervene in the metabolic regulation of the coronary circulation (271).

The effects of acetylcholine seem to be essentially linked to the bradycardia and reduced arterial pressure produced by the drug (29). Thus, in

general, a diminution in the coronary flow is observed following acetylcholine administration. Only with intra-arterial administration has a clear vasodilating action of the parasympathetic mediator been described (103).

To the vast group of coronary vasodilators, new compounds have been added (66, 293, 315, 326, 354). It is important from a practical point of view to study the effects of these substances on the oxygen saturation of the coronary venous blood. Thus it has been observed that papaverine and numerous theophylline and purine derivatives give rise to a direct coronary vasodilation partially or completely independent of stimulant effects on myocardial metabolism (246, 272). The coronary dilating actions of all these compounds is in general limited by the accompanying systemic hypotensive effects (177, 246, 272). From a theoretical point of view it appears that copper ions participate, through a mechanism still undefined, in the phenomena of coronary constriction and that interference with possible coronary receptors by copper ions is the basis of the coronary vasodilator action of many compounds (200).

The cerebral and retinal circulation.—The study of the cerebral reactivity to drugs presents considerable difficulty both because of the physiological characteristics of this vascular region and of technical problems. As is the case with all organs with high functional importance, the brain possesses extremely efficient circulatory autoregulation mechanisms (309) which tend to maintain a cerebral blood flow adequate for the functional activity of the brain (130, 137, 353). From the technical point of view, an almost unique situation in the field of the pharmacology of the circulation is here encountered. The work generally accepted is that carried out in man by the method of Kety & Schmidt (205, 206), while in the experimental animal, where the same method can only be applied with difficulty, the use of new techniques does not permit definite comparisons to be made between the results of the different authors. In the strictly experimental field many factors may intervene which alter cerebral vascular reactions, such as the type of narcosis and the trauma following comprehensive surgical dissections very often carried out for the purpose of isolating the intracranial from the extracranial circulation. This has been proved by a series of experiments based on the measurement of the peripheral jugular pressure which has been found to be a useful method for the study of cerebral vascular reactivity (55, 72, 396). While in the chloralosed dog in good experimental condition the cerebral circulation responds to epinephrine and norepinephrine by vasoconstriction, it is refractory to the vasoconstrictor action of these two amines when the animal is under barbiturate narcosis, is in a condition of respiratory acidosis, or has undergone extensive surgical intervention (55). Deep chloralose narcosis, the type preceded by an initial stimulatory phase of convulsive type, may also lead to this refractory state which is generally associated with very low values for the cerebral flow. This phenomenon may be interpreted as a means of protection against dangerous reductions in the cerebral flow to below critical values. Confirmation of this is found in other experimental

work (133, 342) and also in the clinical observations which have shown the disappearance of the constrictor action of norepinephrine when the patient is suffering from critical circulatory conditions (286). The absence of a cerebral vasoconstrictor action of the sympathetic mediators in the animal under barbiturate anesthesia also finds confirmation in recently published results (70, 152, 196) and is difficult to interpret. It is not yet possible to establish whether the increase in the cerebral flow which epinephrine produces under these conditions is a passive phenomenon linked simply to the associated arterial hypertensive response or whether it depends at least partly on an active cerebral vasodilation. Recent research in animals under barbiturate anesthesia suggests that epinephrine can cause a cerebral vasodilatation dependent on the intervention of a nervous compensatory mechanism involving the parasympathetic (70), or linked to neuronal activation provoked by epinephrine (196). The relationship between the cerebral vasodilator effects of the sympathomimetic amines and their action on the functional activity of the brain have again recently been emphasized by investigations carried out on man (327). It is probable that the action of the sympathetic hormones on the cerebral circulation may depend on the state of cerebral functional activity. Thus strychnine, which is a classical antagonist of the barbiturates, can often cause reappearance or potentiation of the cerebral vasoconstrictor action of epinephrine and norepinephrine (55, 72). The cerebral vascular action of LSD also seems to depend on the state of narcosis (197). These experimental facts demonstrate that in the study of the responses of the cerebral circulation to drugs one must take into consideration the state of functional activity of the brain and the possible modifications thereof induced by the active substances. Although the relation between cerebral functional activity and metabolism have been the object of numerous studies (89, 156, 303, 325), it is not possible to state which metabolic factors govern the cerebral circulation. The availability of oxygen (137) and the production of carbon dioxide and metabolic acids have been particularly considered, but it is not possible to exclude the intervention of other factors such as the ionic displacements which accompany the neuron activation (98). Thus it is not possible at present to explain the clinical observations that in some cases the existence of a normal cerebral blood flow is noted during the course of anesthesia or barbiturate poisoning despite the clear reduction of the cerebral oxygen consumption (32, 287), whereas in other cases a diminution in the cerebral blood flow and in the oxygen consumption has taken place (25).

A more consistent action on the cerebral circulation is exerted by the drugs with vasodilator action. Particularly active are histamine and 48/80 (396), whereas some of the sympatholytics provoke a modest and in general transitory vasodilation (55). The increase in the cerebral blood flow after the administration of strychnine is probably due to the action which this substance exerts on the cerebral functional activity and on cerebral metabolism, according to work undertaken by the present authors. Particu-

larly interesting is the prolonged state of vasodilation induced by strychnine or 48/80. Under these conditions not only the cerebral vasoconstrictor actions of epinephrine and norepinephrine may more easily be observed, but also the effect of clinical doses of ergotamine (72, 142). These results obtained by the registration of the peripheral jugular pressure have contributed to the problems of headache. In this field, the concept of the intra- and extracranial vascular factors (407) has found fresh confirmation in a series of experiments carried out in man. In normal subjects and in those suffering from migraine, recording of pulsation of the temporal artery by means of a piezosphygmograph has enabled the effect of histamine, amyl nitrite and 48/80 in induced headache, as well as the vasoconstrictor action of therapeutic doses of ergotamine and norepinephrine, to be observed (54, 140).

Before ending this chapter some interesting facts will be recorded concerning the determination of intracerebral pressure (256) and the experimental observation of strict analogy between the reactions of the retinal and those of the cerebral circulations (15, 16). The vessels of the nasal mucosa also appear to give similar pharmacological responses to those obtained in the cerebral circulation (72).

The renal circulation.—A characteristic aspect of this vascular region is the unusually large blood flow, on which probably depends the sensitivity of the renal vessels to constrictor actions and their relative refractoriness to the dilator effects of drugs. Thus it is known that the renal vasoconstrictor action of epinephrine is not reversed by the adrenolytics. Recent research seems to indicate that the sympathomimetic amines with a purely hypotensive action, when injected intra-arterially, also give rise exclusively to renal vasoconstriction (369). From this it may be deduced that the adrenergic renal receptors can only respond with stimulation to the action of the sympathomimetic amines. However, the phenoxyisopropyl derivative of paraoxyephedrine, which is included in the sympathomimetic compounds possessing exclusively vasodilator action, produces a distinct increase in the renal blood flow in the unanesthetized dog (181). It is, however, not possible to compare these results with those previously reported which were obtained in anesthetized animals. The variations in renal vascular reactivity produced by anesthetics are well known (365). With regard to 5-hydroxytryptamine, new data has led to the confirmation of its renal vasoconstrictor action (99, 117, 370), but it is as yet not clear whether the antidiuretic action of the drug depends solely on this vascular effect (1, 370, 389). In the therapeutic field, the use of sympathomimetics with pronounced renal vasoconstrictor action has been discouraged in circulatory collapse (273). Nevertheless, it should be borne in mind that in critical circulatory conditions the renal vessels appear to be less sensitive to the sympathomimetic amines (80, 261, 285).

Another interesting aspect of the renal circulation is its autoregulation which seems to depend more on mechanical than functional phenomena (19, 296). Recently, an interesting interpretation of this was advanced based

on the intrarenal separation of the red blood corpuscles from the plasma (212, 305). The effects of the intrinsic regulation of the renal circulation may be seen in experimental animals and in man treated with ganglioplegics. In spite of the distinct arterial hypotension only slight reductions in the renal blood flow were observed (27, 283, 284). On the other hand, in patients suffering from hypertension a reduction in the arterial pressure to normal values obtained with ganglioplegics provoked an intense reduction in the renal blood flow (73, 80). According to some authors this phenomenon indicates a deficit in the mechanisms of renal circulatory autoregulation (73, 80, 284, 285); others have suggested that in patients suffering from hypertension some ganglioplegics have renal vasoconstrictor effects (2, 147). In favor of the second interpretation is the fact that hypotension induced by other substances in patients suffering from hypertension is not accompanied by an equally pronounced fall in the renal blood flow (2, 147).

The mesenteric circulation.—The variations in the circulatory state of this region are those which most decisively affect the arterial pressure. Thus all drugs possessing hypotensive or hypertensive action of a peripheral nature produce mesenteric vasodilation or vasoconstriction respectively. The pharmacological observations recently published in this field would fit into this generalization (96, 385). In the mesenteric area the pharmacological behavior of the splenic circulation has very particular characteristics. The capacity which the spleen has to accumulate large quantities of blood and to contract actively in response to certain drugs with vascular action explains the different effects on the splenic blood inflow and outflow produced by epinephrine and norepinephrine (299). It has also been confirmed that in this vascular region it is possible to obtain an inversion of the vasoconstrictor effect of epinephrine with adrenolytics (299). When the spleen is perfused with Tyrode's solution a vasoconstriction and splenocontraction has been observed not only with epinephrine, but also with acetylcholine (132). The interpretation of these findings is not clear owing to the particular techniques employed and the difference between these results and those obtained by the application of more physiological techniques.

The hepatic circulation.—This circulation is characterized by the presence of two vascular systems (hepatic and portal) having different functional significances. The problems concerning the techniques of study and the physiology of the hepatic circulation have recently been reviewed (192). The pharmacological data concern the vasoconstrictor action which epinephrine and norepinephrine exert either on the circulation of the liver considered as a whole (151), or on the portal and hepatic circulations considered individually (95, 193). Between the two circulatory systems a particular relationship exists. The injection of epinephrine into the hepatic artery also gives rise to its characteristic effects on the portal circulation (58, 95) whereas, when injected into the portal vein, epinephrine does not modify the hepatic arterial pressure (95). The hepatic veins present particular reactivity as shown by constriction, not only by the sympathetic mediators but also by

histamine and acetylcholine (193). To this action may probably be attributed the increase in the mesenteric venous pressure on intravenous injection of epinephrine, which has frequently been observed by the present authors in the anesthetized dog (unpublished observations).

The pulmonary circulation.—The effects of drugs with vascular action on the pulmonary circulation are qualitatively analogous to those which they display on the systemic circulation. Thus in the group of the sympathomimetics, epinephrine and norepinephrine produce pulmonary vasoconstriction (13, 330) and isuprel gives rise to vasodilation (13). The intense pulmonary vasoconstrictor action of 5-hydroxytryptamine has been confirmed (311), and is independent of the bronchoconstrictor action (217). Acetylcholine provokes pulmonary vasodilation (171, 330).

The importance of hemodynamic factors in the genesis of pulmonary edema has lately been confirmed in reviews on this problem (104, 397) and in experimental observations. From these it appears that epinephrine does not provoke pulmonary edema in rabbits in anaphylactic or histamine shock (237). One deals with hemodynamic phenomena which involve or affect the entire cardiovascular system and which have repercussions on the pulmonary circulation (104, 397). Other factors are certainly involved among which neurogenic factors have lately been emphasized (74). The difficulty of establishing the genesis of experimental pulmonary edema, and the uncertainty about the analogies which may exist between this and the edema in human pathology, make it difficult to interpret the therapeutic actions which some substances possessing vascular activity, such as the ganglioplegics (112) and chlorpromazine (230), exert in pulmonary edema of man.

The terminal circulation.—The theory which attribute the vasodilatory action of epinephrine to its glycolytic effects has already been mentioned. This hypothesis has been advanced particularly for the muscular vessels where it has however been shown that adrenolytic vasodilation depends only in part on the increase of lactic acid in the blood (22). Other experimental results attribute the muscular vasodilation provoked by epinephrine to a diminution in the sympathetic tonus which does not derive from a stimulation of the carotid sinus or cardioaortic baroreceptors but from a central sedative or ganglioplegic action of the sympathetic hormone (202). An analogous ganglioplegic action of norepinephrine would explain, according to Dunér & von Euler (109), some of the hemodynamic effects of this amine. It is however certain that, independently of these metabolic and neurogenic phenomena, epinephrine does exert a direct dilating action on the muscular vessels (60). In man 5-hydroxytryptamine produces on this region qualitative effects equivalent to those given by epinephrine (46).

The reactivity of the cutaneous vessels to the sympathomimetic amines has lately been studied. The absence of significant dilator responses to the amines possessing prevalently inhibitory action has led to the conclusion

that the cutaneous circulation only reacts to the stimulant actions of the sympathomimetics (153). In this vascular area a strict analogy also exists between the actions of epinephrine and those of 5-hydroxytryptamine (46). Some studies on the cutaneous vascular action of chlorpromazine have contributed to the demonstration of a central vascular action of this drug. Intravenous injection of chlorpromazine in man produces a cutaneous vasodilation more pronounced than that observed with intra-arterial administration (144). It has also been observed that chlorpromazine inhibits the cutaneous vasoconstriction produced by norepinephrine more intensely than that produced by epinephrine (108).

A problem as yet little studied from the pharmacological point of view is that of the arteriovenous anastomoses which are particularly numerous in the cutaneous circulation (91). By the use of histochemical methods the high content of specific cholinesterases in the cutaneous arteriovenous anastomosis of man has been demonstrated (195) and hence these must be under cholinergic control. Some pharmacological studies of the arteriovenous anastomoses in the femoral circulation of the dog have demonstrated a lack of sensitivity of these vessels to the drugs studied. Only epinephrine appeared capable of reducing appreciably the circulation in them (318, 319).

The fetal circulation.—The physiological characteristics of this circulation have been especially analyzed (3, 49, 50, 170). The effects of epinephrine have also been investigated. The placental vessels do not respond to the sympathetic mediator which only produces passive modifications in the umbilical flow (94).

CONCLUSIONS

A rapid review of the research in the field of the agents active in the peripheral vascular system would suggest that pharmacology may actually be considered as the meeting point between chemistry and biochemistry on the one hand and physiology and therapy on the other.

It may be recorded that 1956 was the centenary of Vulpian's observation on the color reaction of ferric chloride which demonstrated the presence of epinephrine in the suprarenals, and that 1957 is the fiftieth anniversary of the synthesis of histamine by Windaus & Vogt.

In the field of the chemical mediators the present investigations have led to the recognition of in addition to the mimetics and antagonists, drugs inhibiting synthesis and destruction, and liberators which form a logically linked series leading to the competitive and sensitizing agents of the inhibitors and potentiators.

The continuous introduction of new synthetic derivatives has represented a noteworthy stimulus to the study of the possible mechanisms of action of products already known.

As regards the precise analysis of the effects of each of these new drugs on the various vascular districts, much remains to be done owing to the

diversity in the reactions of different animal species, the interference caused by anesthetics, and in general the influence of the environmental conditions imposed by the different techniques. From this point of view, pharmacological research carried out clinically, of which only slight mention has been made as it is outside the scope of this review, is assuming particular importance. The authors would like to express the hope that the development of techniques will enable a more definite forecast of clinical results to be made on the basis of animal studies, and also an accurate analysis in man himself of the more precise mechanisms of therapeutic effects.

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KIDNEY, WATER AND ELECTROLYTE METABOLISM¹

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During the period covered by this article there have appeared a number of noteworthy reviews and monographs concerned with the kidney and with water and electrolyte balance. Some deal with these in clinical practice (1 to 6); others are more basic. Welt has edited a special number of the *Yale Journal of Biology and Medicine* as a memorial volume to John P. Peters (7). This volume has also been brought out in book form (8). It contains original articles by a group of Peters' former students and is a rich and up to date source book for students interested in recent work in the fields of acid-base balance and water and electrolyte excretion. Winton had edited a collection of articles from workers past and present at University College, London. This volume (9) comprises ten lectures given at University College, London in the summer of 1955 and commemorates Cushny's "modern theory" some forty years after he enunciated it. It contains a valuable series of statements on several aspects of modern renal physiology by a remarkably productive school who have continued in Cushny's tradition of careful work on clear ideas. One of this group, E. J. Harris, has also written a monograph (10) which presents a succinct and up to date statement of the present knowledge of movement of inorganic and simple organic substances across cell membranes. *The British Medical Bulletin* published an issue this year (11) devoted to papers on "Physiology and Pathology of the Kidney."

In the perplexing and rapidly developing field of ion and water regulation in cells there have appeared several excellent reviews; outstanding among them are Conway's (12) and Lowenhaupt's (13). Since the author of the present review does not feel competent to assess critically the present status of this field of ion and water regulation in tissues, he will not attempt here an exhaustive review of the subject. He refers the reader to these more appreciatively written reviews, leaving his consideration of electrolyte regulation to a few studies in the field as they bear on general problems of acid-base balance, water and osmoregulation that will be considered below.

The study of renal physiology at this time appears to be chiefly concerned with autoregulation of renal circulation and renal metabolism as it relates to regulatory and transport functions. The present review will address itself primarily to this later area. The Pappenheimer-Kinter ideas on autoregulation of renal circulation (14) have been carefully reviewed by Bradley (15) and Winton (16). A discussion of some of the current evidence contradictory to the theory will be presented.

¹ The survey of literature pertaining to this review was completed in June, 1957.

This review does not pretend to be exhaustive or complete. The author, confronted with the mass of literature evolving month by month, has decided quite arbitrarily to limit the bulk of his discussion to certain areas of the subject that seem to him to be its central issues at the moment and to relate those issues to past work and possible future lines of development. Having thus decided on the scope and nature of the review, he apologizes to those whose work has possibly been overlooked in its preparation.

TRANSPORT MECHANISMS

When substances move across biological membranes against gradients of osmotic pressure, diffusion, or chemical potential, we refer to this as "active transport." It has been the subject of several recent symposia and books (17, 18, 19). Research workers are looking for "carriers" either on or within cells, carriers that exist in fixed amount and operate at known rates. The nature and amount of some of these carriers have been studied (20, 21, 22). Besides the characterization of carriers, an understanding of active transport mechanisms requires knowledge of the energy source for the process and the way that energy is coupled with the carrier system. This entails studies of biological oxidations and their coupling with phosphorylation, enzyme kinetics, a study of model organic reactions and the correlation of steady state data from tissue metabolism with *in vivo* observations of the transport mechanism operating under a variety of experimental conditions.

In the kidney, transport mechanisms have been extensively studied, both reabsorptive and excretory. Although the detailed nature of none of these mechanisms is known, they have come to be classified by certain specific characteristics displayed by a single substance or a group of substances. Thus there is excretory transport for a number of organic acids: *p*-aminohippurate, Diodrast, phenol red, and penicillin. All these substances may share one "mechanism" in that each mutually inhibits the transport of the other and each is inhibited by probenecid (Benemid) (23). In like manner a group of organic bases is also excreted by the kidney tubules, and their several mechanisms share certain characteristics in common which justify the loose descriptive phrase, "organic base mechanism." This "mechanism" is characterized by the fact that it transports, among other substances, tetraethylammonium (24) and N-methylnicotinamide (25) and is inhibited by the basic dye, cyanine (No. 863) (26) in small concentrations. Furthermore it is Benemid-insensitive and not inhibited by members of the *p*-aminohippurate group (27).

On the side of reabsorptive transport there are likewise single substances and groups of substances showing certain common characteristics of mechanism. Certain groups of amino acids compete with one another for reabsorption (28); this is also the case for creatine and glycine (29). The ions phosphate, sulfate, and acetoacetate are related in reabsorptive transport in that each is accelerated by phlorizin and depressed by glucose (30). Thus we see a certain kind of group specificity in transport. This does not mean that the mechanisms transporting each member of a group are identical; it only

means that whatever the individual mechanisms are they share common characteristics at some point. To know even this is helpful for further clarification because it at least allows for a generic classification of groups of mechanisms and thus includes or excludes certain possibly fruitful lines of deeper experimentation.

With this general background description, I should like now to consider some recent research on several of these transport mechanisms.

TUBULAR EXCRETION OF ORGANIC BASES

The elimination of a number of organic bases by the kidney has been shown to involve not only glomerular filtration but tubular excretion as well. Sperber was the first to demonstrate the tubular excretion of the quaternary base, N-methylnicotinamide in the chicken (31). Beyer and his colleagues subsequently showed in 1950 that N-methylnicotinamide was also thus handled by the kidney of the dog (25). The same excretory characteristics were shown for tetraethylammonium by Rennick *et al.* in 1954 (24) and for mepiperphenidol (Darstine) by Beyer *et al.* in 1953 (32). Orloff, Aronow & Berliner (33) in 1953 showed that the nonquaternary base 2-benzyl-2-imidazoline (Priscoline) is also excreted by the renal tubules. As was mentioned earlier, these organic bases differ in their tubular transport from the organic acids such as *p*-aminohippurate (PAH) in that they are not inhibited by Benemid or any of the organic acids themselves. Furthermore, as was shown by Peters *et al.* in 1955 (26), N-methylnicotinamide excretion is exquisitely sensitive to small quantities of the basic cyanine dye (No. 863) which, while inhibiting the organic base transport, is without effect on that of PAH.

Interesting research has continued to appear in this field. Rennick, Kandel & Peters (34) have recently shown that both tetraethylammonium and N-methylnicotinamide are excreted by the tubules of the dog and chicken and that the tubular excretion of both these bases is markedly inhibited by the basic cyanine dye (No. 863). Further evidence of the interrelatedness of these two excretory mechanisms comes from the fact that they mutually inhibit one another. It is interesting and significant that these authors observed a much higher concentration of the cyanine dye in the kidney than in other organs studied. Thus the kidney apparently selectively concentrates the dye. Further attempts to localize the site of the dye within the kidney cells revealed it to be in the mitochondria almost exclusively. Since the mitochondria house the enzymes of most of the reactions of oxidation and coupling with phosphorylation, it would seem to be a fruitful research to look for an effect of the cyanine dye on oxidation and coupling with phosphorylation in mitochondria from kidney cortex cells. Certain other dyes, such as methylene blue, are known "uncouplers" of oxidation and phosphorylation; perhaps a study of the effect of cyanine on these reactions could give a lead to the mechanism of action of the cyanine inhibition of organic base transport.

There have been important studies this year attempting to understand

these organic base transport systems with *in vitro* preparations. Using the Cross & Taggart technique of studying transport *in vitro* (35), Farah & Rennick (36) have compared, under a variety of conditions, the uptake of tetraethylammonium and PAH by dog kidney cortex slices. Alpha-ketoglutarate, succinate, and octanoate, while markedly inhibiting PAH uptake, had essentially no effect on that of tetraethylammonium, confirming previous observations by the same authors in the intact dog. Study of the effects of cyanide, azide, sodium fluoride, iodoacetic acid, and 2,4-dinitrophenol showed that each of these inhibitors blocked the *in vitro* transport of both tetraethylammonium and PAH. Several other inhibitors, such as malonate, fluoroacetate, dehydroacetate, and phlorizin, which the authors differentiate as "Krebs cycle inhibitors," showed the well-known block of PAH uptake but were without effect on tetraethylammonium. They conclude that the transport mechanisms for tetraethylammonium and PAH are different on the basis of their metabolic requirements; the tetraethylammonium mechanism can continue to function after blocking the Krebs cycle by a number of inhibitors which depress PAH transport. "It is thus likely that the tetraethylammonium transport mechanism obtains its energy supply from a source other than the Krebs cycle." Such a conclusion, based on studies with a group of inhibitors whose effects in the fairly well organized tissue are certainly not that specific, seems to me somewhat dangerous. Phlorizin and antimycin A cannot be considered primarily inhibitors of the Krebs cycle but rather affect sites in the terminal electron carrier system with secondary effects in the cycle. Indeed, Krane & Crane (37) have recently shown that phlorizin inhibits the uptake of D-galactose-1-C¹⁴ by kidney slices in a way that they interpret as interference with diffusion of the sugar into the cell, a phenomenon quite independent of effect on oxidative metabolism or the "active" transport process. Furthermore, phlorizin in the concentrations used in the Farah, Rennick experiments has not been shown to depress respiration or the Krebs cycle in slices. Thus in Farah & Rennick's studies (36) the differing results obtained with a variety of inhibitors on PAH and tetraethylammonium transport in slices must be interpreted with caution in terms of differing energy sources for the transport mechanisms until it is carefully determined whether the inhibitors in their experiments were actually disrupting respiratory energy metabolism or rather blocking some nonrespiratory carrier system either in or on the cell. One recalls experiments like those of Foulkes (21) where azide, a potent respiration inhibitor, strongly blocks potassium transport in yeast in concentrations where oxygen consumption is unaffected. Here the azide may be binding a surface carrier, heme in nature, which has nothing to do with the oxidative energy-producing reactions of the cell.

Farah, Rennick & Frazer (38) have studied the effect of a number of organic bases on tetraethylammonium transport in the dog renal cortex slices. Some of these bases are themselves excreted by the tubules. Priscoline, guanidine, methylguanidine, piperidine, and N-methylnicotinamide in-

hibited tetraethylammonium uptake, but in the concentrations used had little effect on uptake of PAH. These findings additionally support the fact that tetraethylammonium and PAH transport are different in the kidney. Tetramethylammonium bromide did not interfere with tetraethylammonium uptake by the slices, indicating either that tetramethylammonium is not excreted by the tubules or, if it is, that its affinity for the mechanism is extremely low and inhibitory effects cannot be demonstrated. The demonstration *in vitro* that one base inhibits the uptake of another suggests that the authors are dealing with a competitive type inhibition where the several bases compete for the carrier system on the basis of substrate-enzyme specificity. It would be useful, perhaps, to carry out some initial kinetic studies (which, of course, would be crude because one would be dealing with a multienzyme system conditioned by permeability factors and a complex steady state in the intact cell). Nevertheless if one were to relate inhibitor concentration (here a second organic base) to reaction velocity (uptake of first organic base) in a rough Burke-Lineweaver plot, one might get some idea of the nature of the inhibition and the relative affinities of the various bases for the "carrier" system. Further studies then on more carefully isolated single-unit systems might ultimately lead to a more careful description of the exact nature of the whole base transport mechanism.

Finally Farah & Frazer have published observations (39) on the influence of some alcohols on the uptake of tetraethylammonium by the dog kidney cortex slices. Uptake was increased by all the alcohols studied; the longer the chain length of the alcohol the lower the concentration needed to produce 40 per cent increase in uptake. This alcohol-induced stimulation of tetraethylammonium uptake was not affected by fluoroacetate or malonate but could be inhibited by tetraethylthiuram disulfide (Antabuse). Since none of the alcohols can be tolerated by the intact dog, it was impossible to correlate these *in vitro* findings with any similar effects of the same alcohols on tetraethylammonium excretion by the tubules of the intact animal. Therefore, at the moment the exact meaning of these interesting *in vitro* findings remains obscure.

In relation to the series of studies discussed above one must also consider the important work in organic base transport coming from Shideman's group at Wisconsin. Recently Le Sher & Shideman (40) have presented observations on the metabolic characteristics of the renal tubular transport of Darstine which Beyer has shown is excreted by the tubules of the dog (41). Again studying uptake of Darstine by dog cortex slices, the authors have investigated the effects of a variety of inhibitors, substrates, and PAH on the process. They conclude that the Darstine transport system differs from both those for PAH and N-methylnicotinamide because Darstine uptake is unaffected in the presence of PAH, N-methylnicotinamide or carinamide. Transport mechanisms for both PAH and Darstine were felt by the authors to derive a significant portion of their energy requirements from the Krebs cycle. Here again, in the absence of respiratory measurements or careful

kinetic studies, conclusions about "energy requirements" have been drawn without the inclusion of permeability and surface carrier possibilities in the interpretation of the results. The type of study represented here, and in the foregoing papers, is to be encouraged because only by using a wide variety of organic bases with differing molecular configurations can we begin to erect meaningful theories about the specificity characteristics of these intriguing transport systems. One can point to a study such as that of Rosenberg & Wilbrandt (42) (which will be discussed below) in this regard. They substituted the hydroxyl groups in the phloretin molecule and studied the effects of these substitutions on the capacity of the molecule to inhibit glucose transport across the erythrocyte membrane. Their studies led them to interesting theoretical conclusions about the nature of the phloretin-carrier reaction. Such studies should now be applied to the organic base transport problem using representatives of the Darstine and tetraethylammonium and N-methylnicotinamide groups for intramolecular substitutions.

Baer *et al.* (43) have studied the renal excretory characteristics of a new ganglionic blocking agent, 3-methylaminoisocamphane hydrochloride (Mecamylamine). These workers have found that the rate of excretion of Mecamylamine is greatly affected by the pH of the urine, and in clearance studies they have shown that when the urine is acid the Mecamylamine is excreted by glomerular filtration plus a high rate of tubular excretion. Indeed, under these conditions the renal extraction is so complete that the Mecamylamine clearance measures effective renal plasma flow as well as the PAH clearance at low plasma levels. On the contrary, when the urine is made alkaline either by the infusion of sodium bicarbonate or the administration of acetazoleamid (Diamox), Mecamylamine excretion is markedly reduced and then one measures glomerular filtration and net tubular reabsorption of the substance, the Mecamylamine clearance going well below the simultaneously determined clearance of creatinine. When the urine is acid and Mecamylamine is being excreted by tubules, this excretion is unaffected by PAH or Benemid. The authors speculate about the reasons for the marked change in net tubular transport with changes in urinary pH. They wonder whether it can be explained on the basis of the Orloff & Berliner theory (44) which attempts to explain the effect of urinary pH on the excretion of ionizable compounds. These authors assume (a) only one ionic species is appreciably diffusible and (b) diffusion is sufficiently rapid that equilibrium is attained, or nearly so, between the tubular urine and blood. The Mecamylamine findings cannot be explained on the basis of such a theory because at pH 7.0 one molecule of Mecamylamine in 25,000 exists as the base, while at pH 8.0, 10 in 25,000 are present as the base. Yet over this pH range the net tubular transport changes completely from excretion to reabsorption. One wonders whether the total excretory process for Mecamylamine involves bidirectional tubular flux with one direction sensitive to changes in the bicarbonate-hydrogen ion system. What is the effect of potassium loading on Mecamylamine excretion? What happens to its excretion in potassium de-

iciency with the alkalosis of that condition? What is the effect of respiratory alkalosis? Furthermore one wonders about the relation between the tubular excretory mechanisms for Mecamylamine and those of some of the other organic bases discussed above. Do tetraethylammonium, N-methylnicotinamide, Darstine, and Priscoline themselves show changing net tubular transport with changing urine pH and do they affect either the excretory or reabsorptive transport of Mecamylamine? These and many other related questions come to mind as one thinks of lines of further research in this field of organic base excretion.

ORGANIC ACID EXCRETION

Studies discussed in the section above have shown how clearly the tubular transport of organic bases is differentiated from organic acids such as PAH. Correlative studies observing PAH uptake from buffered saline media by slices of kidney cortex under a variety of experimentally imposed conditions and PAH secretion by the kidney *in vivo* under similar conditions were pioneered by Forster & Taggart (45), Taggart & Forster (46) and recently extended by Forster & Copenhaver (47). These studies lead to the conclusion that the active transport of PAH from peritubular blood to tubular lumen derives its energy from the high energy phosphate bonds formed during the respiratory metabolism of the cell. Further studies lead to the speculation that during its transport PAH is hydrolyzed to *p*-aminobenzoate and glycine and subsequently resynthesized to PAH (48). Crucial experiments on the excretion of PAH labelled with C^{14} in the carboxyl group indicated, however, that such hydrolysis and resynthesis was not a possible mechanism (48). Further theoretical speculation led to the idea that it was the carboxyl group on the PAH which interacts with the cellular carrier in the transport mechanism. This postulated mechanism involved the function of coenzyme A in the synthesis of intermediates in the chain of transport reactions. In a recent brilliant experiment (49) Taggart put his own carboxyl mechanism of PAH transport to a severe and crucial test. In an attempt to determine the nature of the chemical bonding between carboxyl group and carrier, he employed PAH labelled in the carboxyl group with O^{18} . Taggart postulated that information concerning the extent of the loss or retention of the O^{18} in the PAH during its transport should set limits to the type of mechanism involved. Accordingly the O^{18} -labelled PAH was infused slowly directly into the renal artery of the left kidney of a dog. Urine was collected from the cannulated left ureter. Renal function studies done at the end of the infusion period revealed that about 35 per cent of the PAH infused into this kidney had been added to the urine by glomerular filtration and 65 per cent by tubular excretion. The PAH collected in the urine was recovered, degraded and the position of its O^{18} determined; it was shown quite conclusively that tubular excretion caused no appreciable loss of O^{18} from the carboxyl group. Since this experiment conclusively excludes certain types of covalent bonding between carboxyl group and carrier, it was necessary to conclude that

the carboxyl group cannot participate in PAH transport in the manner originally postulated. The O^{18} data are considered more consistent with an ionic mechanism ($-\text{COO}-\text{R}^+$) and suggest to Taggart the possibility that PAH transport may involve an ion exchange mechanism such as has been postulated for hydrogen and potassium ions. It is certainly brilliantly conceived and carefully executed definitive experiments such as these that are needed to advance our thinking. Although negative, in that it disproved a good working hypothesis, the O^{18} experiment of Taggart cleared the field for new ideas and pointed the way to the type of crucial experiment we are required to perform before really definitive understanding of a transport system is available.

Knoefel & Huang (50) have presented an interesting study of protein binding, toxicity, *in vitro* uptake by dog kidney slices, and *in vivo* excretory characteristics in the dog of benzoic acid and fourteen iodinated benzoic acid derivatives. There was a high correlation between the extent of protein binding of a compound and its uptake by kidney *in vitro*, suggesting that "uptake" *in vitro* might be a function of protein binding rather than "transport." In the intact kidney of dog it was found that benzoic acid and ten of its derivatives are reabsorbed by the tubules from the glomerular filtrate. Three of these derivatives which were found to be reabsorbed to a lesser extent had greatly different acidity and solubility but showed relatively little protein binding. Benzoic acid itself, showing no protein binding, is extensively reabsorbed. The authors wonder whether protein binding somehow determines tubular reabsorption or excretion. (Below is discussed another aspect of this problem.) Four of the compounds were shown to be excreted by the tubules. These compounds had an acylamido group in the 3, the 4, or the 3,5 position. An acylamido group in the 2 position did not lead to tubular excretion. Three of these acylamido-substituted compounds exhibited an excretory T_m (4-acetamido-3,4-diiodobenzoic acid, 3,5-diacetamido-2,4,6-triiodo, and 3,5-dipropionamido-2,4,6-triiodo). These three compounds are apparently transported by the same mechanism. These interesting observations were presented by the authors without much speculative comment concerning the significance of a critical molecular configuration of substituted benzoic acids for transport in one direction or the other across the tubule. Using an increased number of such substituted compounds in combined *in vitro* and *in vivo* experiments one could perhaps arrive at a more definitive hypothetical formulation of necessary molecular structure. This knowledge could then lead to experiments such as those of Taggart (49) discussed above where the use of labelled compounds with an analysis of distribution of isotope in excretory products or inside the cell might lead to definition of the bonding with the carrier or the nature of the intermediary reactions of the transport system.

Some of the factors of tubular transport conditioning the thinking of Knoefel & Huang's work (50) also entered an interesting piece of research on organic acid transport recently reported by Ochwaldt & Pitts (51). It has

long been known that phenol red and Diodrast are excreted by a similar tubular mechanism; however, the phenol red clearance is always well below that of Diodrast. They pointed out that the difference in clearance might be due to available substrate energy (as evidenced by the fact that acetate increases the rate of excretion of both substances). They further postulated that the degree of protein binding might predicate the differing transport rates. [Certainly this was seen as a factor in Knoefel & Huang's studies (50)]. In a series of ingenious experiments the investigators were able to show that the availability of acetate is not a significant factor in determining the relatively low phenol red/Diodrast clearance ratio. In contrast, however, high protein binding of phenol red is, indeed, a factor contributing to the low ratios observed normally. Plasma proteins were progressively lowered during the course of dog clearance experiments by plasmaphoresis where dextran was substituted for plasma in a stepwise fashion. Although plasma protein decrease certainly raised the phenol red clearance, it did not abolish completely the difference between phenol red and Diodrast clearances; therefore, there are other as yet unknown factors operating to dictate the differences. Certainly among these might be differing specificities of substrates and carrier sites and differing velocity constants in the reaction between reactant and carrier. Although not definitive in answering the original question posed, these were elegant experiments and certainly open the way for a new approach to this kind of problem where one wants to vary *in vivo* several factors operating where a group of substances is handled by "one" transport mechanism.

Tricarboxylic acid cycle in kidney.—In 1953 Craig *et al.* (52) reported that the infusion of succinic or α -ketoglutaric acids in dogs resulted in the excretion in the urine not only of the infused acid but of malic acid also. Furthermore the malic acid excreted exceeded that passing through the glomeruli; thus there was a net tubular excretion of malic acid under these circumstances. The administration of sodium malonate, the competitive inhibitor of succinic dehydrogenase, abolished this excess malic acid getting into the urine by tubular excretion, presumably by blocking the synthesis of malic acid from either succinate or α -ketoglutarate in the tubular cells. Recently Vishwakarma² (53), working in the author's laboratory, has repeated and extended the above findings of Craig and Miller. Citrate, α -ketoglutarate, and succinate will, when infused into dogs, result in measurable plasma levels of malic acid which is then excreted by glomerular filtration and net tubular secretion (the malic here probably having been synthesized in the tubules from the infused substrate). When, during the infusion of any one of these three substrates, sodium malonate is administered, not only is the net tubular secretion of malic abolished, but one now sees a glomerular filtration and net reabsorption of malic acid. Thus there appears to be a bidirectional

² The research described here has been supported by grants from the American Heart Association and A-1381 from the U.S. Public Health Service.

flux of malic acid across the tubules, one limb of which (the secretory) is inhibited by malonic acid. Such a supposition is strengthened by experiments in which L-malic acid itself or its immediate precursor, fumaric acid, is infused. Here one is beyond succinic dehydrogenase in the tricarboxylic acid cycle and therefore would eliminate the likelihood of malic acid synthesis in the tubules from a substrate behind the succinic dehydrogenase system. Indeed, when one infuses either sodium L-malate or sodium fumarate one gets an easily measurable plasma malic acid level, and during infusion of both these substrates one measures a glomerular filtration and net tubular reabsorption of malic acid; no net tubular secretion is seen. Furthermore this tubular reabsorption of malic acid is unaffected by malonic acid as one would expect.

When sodium fumarate is infused at a rate sufficient to produce filtered loads of malate equivalent to those produced during sodium L-malate infusion, there is observed a striking difference in the amount of the filtered load that is reabsorbed. During malate infusion maximum malate reabsorption was only 15 to 25 per cent of the filtered load; while during fumarate infusion, malate reabsorption was increased to 50 per cent or more of the equivalent filtered load. In seeking an explanation for this difference, Vishwakarma wondered whether fumarate could be "sparking" malic acid reabsorption much in the same way that it "sparks" oxidation in the tricarboxylic acid cycle in tissues. Accordingly experiments were done in which maximum malic acid reabsorption was established with the infusion of sodium L-malate. Then sodium fumarate was added to the infusion in small catalytic amounts. This addition of fumarate in amounts much too small (1/60th the amount of malate) to affect the malate load itself, strikingly increased the rate of malic acid reabsorption some two to three times.

Thus these experiments with just one of the intermediate substrates of the tricarboxylic acid cycle show that in several respects, at least, factors that affect the operation of that cycle in tissue oxidation also affect the tubular transport (and synthesis) of one of its intermediates. The intriguing possibility thus presents itself that the ubiquitous oxidative cycle in tissues is adapted as a transport mechanism of its own intermediates in the kidney.

INORGANIC PHOSPHATE TRANSPORT

It is well known that inorganic phosphate, which is freely filterable from plasma, is excreted by combined processes of glomerular filtration and tubular reabsorption in the dog (54) and man (55) and that in these two species there is a maximal phosphate reabsorptive rate (Tm_{PO_4}). In the cat no such Tm_{PO_4} has been observed (56). Just this year it has been shown that inorganic phosphate is excreted by the tubules of the chicken (57). Phosphate is of intrinsic interest not only as an electrolyte in the body, but because it plays such an important role in the whole of intermediary metabolism. Thus its transport in the kidney provides us with a unique opportunity to study a transport mechanism as it operates in the regulation of an important

metabolite. One asks the question at this point: "To what extent might the intermediary metabolism of phosphate operate in the mechanism that transports it? Does phosphate enter into or use some of its own intermediary reactions in its transport?" Definitive answers to these questions are not before us, but the question itself is, as one contemplates future research in this field.

Important work has appeared in recent months bearing on the phosphate transport problem, particularly as it is affected by parathyroid hormones and changes in acid-base balance.

Parathyroid and phosphate reabsorption.—It has been realized for some time that the parathyroid glands exert some control over the excretion of phosphate. Whether this is a direct effect on renal tubular reabsorption of phosphate or a response occurring secondarily to an effect on bone calcium was for some time not clear. However, now the picture is greatly clarified and it is recognized that the parathyroid secretion does affect renal phosphate reabsorption.

During the few hours immediately following a single injection of parathyroid extract there is a phosphaturia which has subsided by the time blood calcium is at its peak some 10 to 20 hr. later. This initial phosphaturia has been shown to be the result of an increased renal blood flow and glomerular filtration rate caused by the parathyroid extract, as a pharmacological side effect. It is unaccompanied by any change in Tm_{PO_4} (58). Furthermore it was shown in 1948 by Jahan & Pitts (59) that there was also no change in Tm_{PO_4} 20 hr. after two injections of parathyroid extract. In their experiments hypercalcemia was maximum when their PO_4 titrations were done. In 1953 Sirota (60) showed that several patients with parathyroid adenoma showed a marked increase in Tm_{PO_4} following removal of the tumor. This led investigators to wonder whether it is necessary to condition the tubules to a responsiveness to parathyroid by repeated injections over some days before giving the "challenging" shot. This surmise has turned out to be correct, and the evidence for it comes from several sources. Hiatt & Thompson have presented an excellent series of papers on phosphate and calcium excretion in man (61 to 64). These authors found that parathyroid extract will depress Tm_{PO_4} , but prolonged treatment is necessary to demonstrate the effect (61). Hyperparathyroidism was induced in five normal subjects and two hypoparathyroid patients by the administration of 1800 to 2400 units of parathyroid extract over periods of 60 to 120 hr. In all these subjects hypercalcemia, hypophosphatemia, calciuria, and hyperphosphaturia were observed. In all these individuals there was a consistent depression of Tm_{PO_4} without any change in inulin or PAH clearances. In these studies there was no determination of the minimum time of parathyroid treatment necessary to produce the Tm_{PO_4} -depressing effect.

These same authors next showed that the reduction of urinary phosphate that follows the intravenous infusion of calcium in normal individuals (63) is probably due to parathyroid inhibition accompanying the hypercalcemia.

They reasoned that if this were the case three things should be observable: (a) Calcium infusion should be associated with an increased Tm_{PO_4} . This was observed. (b) One would anticipate that alterations in the level of circulating parathyroid hormone would affect the usual response of urinary phosphorus to calcium administration. Accordingly they observed no fall in urinary phosphorus in profoundly hypoparathyroid patients given the calcium infusions. (c) The maintenance of a constant level of circulating parathyroid hormone by daily injections might be expected to abolish the phosphorus excretion changes following calcium infusion. Indeed, this is what happened. Thus their results with calcium infusion are consistent with the idea that the observed phosphate excretion changes were caused by an inhibited parathyroid.

These same authors have studied the effect of prolonged phosphate loading on Tm_{PO_4} (64). They gave repeated daily intravenous infusions of large quantities of phosphate and at intervals determined Tm_{PO_4} . The Tm reductions they observed in their subjects as a result of this prolonged loading were dramatic; for example, in $\mu g.$ per min. 102 to 0, 137 to 59, 110 to 70. The authors do not come straight out and say so, but they lean towards the view that these Tm_{PO_4} reductions result from a stimulation of the parathyroid by the prolonged hyperphosphatemia. This supposition is strengthened by the fact that their most minimal effect was obtained in one severely hypoparathyroid individual. The view is in accord with the work of Foulks (65) who found that he could sensitize dogs to a Tm_{PO_4} -lowering effect of a single injection of parathyroid extract by keeping them on a high phosphate diet. In other words, the continual high phosphate was in fact the same as giving them prolonged parathyroid injections.

These studies with parathyroid and Tm_{PO_4} strongly suggest that there is in the phosphate reabsorptive mechanism some component that is capable of an "adaptive hypertrophy" much in the same way that glutaminase in kidney adaptively increases with prolonged acid loading (66). There should be a most fruitful field for research here, and it seems the next obvious direction in which the parathyroid-phosphate problem should move. One interesting paper has appeared which perhaps represents one sort of approach and which is of great interest itself in this problem. DeVerdier (67), working at Uppsala, has studied the effect of parathyroid extract on the incorporation of P^{32} into the organic phosphorus fractions of rat kidney. Thirty minutes following the injection of parathyroid extract (or saline), the kidneys were removed, a trichloroacetic acid filtrate prepared and the phosphorus fractions separated by ion exchange chromatography. The specific activity was then determined in the several fractions. It was generally noted that the specific activity was greater in the phosphorus fractions from the parathyroid-treated rats, and the author concludes that this is most likely uridine diphosphate or a derivative of it. Perhaps uridine diphosphate is involved as a coenzyme (either directly or indirectly) in the phosphate transport mechanism and its level of activity is under parathyroid (and possibly other

hormonal) control. It would be interesting to see DeVerdier's studies repeated with rats undergoing prolonged parathyroid treatment and in animals on high phosphate diets. It is not carrying speculation too far to suspect that one of the phosphate coenzymes or one of the adenine nucleotide fractions is involved in phosphate transport and that it is susceptible to induced adaptation. Mitchell (22) studying inorganic phosphate transport in *Staphylococcus aureus* has suggested the presence of phosphorylated carriers in the wall of these organisms and postulated that these carriers transport phosphate across the surface of the cell as a carrier-ion complex and then pass it on to adenosine diphosphate within the cell. Similar conclusions were reached by Gourley in his studies of human erythrocytes (68). He suggested the labile phosphorus of adenosine triphosphate (ATP) as a precursor of the cell inorganic phosphorus. This indicated to him that phosphate ions enter the erythrocyte by a process that forms ATP. Since the uridine and adenine nucleotide systems are closely related in cell metabolism, a close look at these systems in phosphate transport seems warranted from likely supposition and previous evidence.

Other hormonal effects on phosphate transport have come to light in the past year. Davidson & Levinsky (57) have shown that phosphate is excreted by the tubule of the chicken kidney and that this mechanism is increased by parathyroid infusion. This preparation should provide a useful means of studying another aspect of the parathyroid-phosphate problem.

Nassim, Saville & Mulligan (69) have studied the effect of stilbestrol on TmPO_4 in three women suffering from post-menopausal osteoporosis, one normal post-menopausal woman and one normal male. These authors claim that there was a depressed TmPO_4 in each case as a result of the stilbestrol. Their effects were not nearly so big as those seen after parathyroid treatment. Indeed, the effects are so small as to be open to question. One would want to see a careful restudy of these effects on animals using careful clearance technique. The authors ascribe their TmPO_4 -depressing effect of stilbestrol to "suppression of an anterior pituitary factor" which they assume to be growth hormone. This study, complicated by method and interpretation, is sufficiently interesting to warrant repetition, extension, and a more penetrating interpretation of the results.

Relation between bicarbonate and phosphate reabsorptive mechanisms.—In an examination of the relationship of phosphate reabsorption during changes in acid-base balance Malvin & Lotspeich (70) have shown, under the circumstances of their experiments, that a variety of conditions causing increased bicarbonate excretion are associated with a decrease in TmPO_4 in the dog. That this is not only the result of alkalosis associated with bicarbonate infusion is shown by the fact that the administration of the carbonic anhydrase inhibitor, Diamox, also causes a depression in TMPO_4 . This agent, while causing a mild acidosis from loss of fixed cation, nevertheless depressed TmPO_4 . Here again an alkaline, bicarbonate-laden urine was the common denominator. The results seemed best explained at the time by postulating

two separate "pathways" of bicarbonate reabsorption. The first and possibly major pathway is one involving the enzyme carbonic anhydrase; the second, one in which bicarbonate is reabsorbed as such. It was postulated that it is along this second pathway that bicarbonate and phosphate compete in transport and that this competition occurs under any circumstance which either saturates the first carbonic anhydrase path (as with bicarbonate loading) or inhibits it (as with Diamox). Under each of these circumstances excess bicarbonate enters the second path where phosphate transport is then interfered with. These assumptions were given added weight by experiments in which TMPO_4 was depressed by producing a respiratory alkalosis (hyperventilation with bicarbonaturia at normal or depressed plasma bicarbonate levels). That the TMPO_4 -depressing effect of both bicarbonate infusion and respiratory alkalosis was completely independent of changes in plasma pH was evident from an experiment in which the dog was infused with bicarbonate but at the same time made to breathe a mixture of 90 per cent O_2 and 10 per cent CO_2 so as to keep pH constant at elevated plasma bicarbonate levels. In this experiment plasma pH averaged 7.29 during control periods and 7.30 during bicarbonate infusion, but phosphate reabsorption dropped from 72 $\mu\text{M}/\text{min.}$ to 52, 46, and then 40 $\mu\text{M}/\text{min.}$

There is yet another interpretation of these data which was not discussed in their paper; that is, the relationship between urine pH and the excretion of weak electrolytes, a relationship that has been discussed by Orloff & Berliner (44) and that was alluded to above when discussing the change in net tubular transport of Mecamylamine with changing urine pH (43). Phosphate is certainly a weak electrolyte; its pK at 6.8 is near plasma pH and thus in tubular urine before its acidification it is fairly evenly divided into acid and alkaline forms. This degree of dissociation would condition not only the diffusion characteristics of the phosphate, but also the kinetics of any carrier complex formation taking place during transport of the phosphate. Therefore to raise the urine pH would alter markedly the predominant form of the inorganic phosphate and thus probably alter these variables of diffusibility and transport kinetics. Thus to discuss the effect of bicarbonaturia on phosphate reabsorption only in terms of a dual pathway for bicarbonate was perhaps to restrict somewhat the possible theoretical interpretation of the results.

Relation of kidney to parathyroid control of blood levels of phosphate, calcium, and citrate.—The studies reported above establish quite definitely that the parathyroids have a primary renal effect on inorganic phosphate. The nature of the parathyroid control of calcium level in extracellular fluid has remained obscure, it being unclear whether changes in blood calcium occur secondarily to the renal mediated changes in blood phosphate or whether they result from a primary action of the parathyroid on bone. It was not until the classic study of Grollman (71) that this problem was clarified. Following nephrectomy the parathyroidectomized dog gradually loses the capacity to increase blood calcium in response to parathyroid extract. Groll-

man showed that if blood levels of phosphate, magnesium, and calcium are maintained within normal limits by peritoneal dialysis, the nephrectomized-parathyroidectomized dog can respond to parathyroid extract with a hypercalcemia, and thus a direct extrarenal hypercalcemic effect of parathyroid can be shown. The hypercalcemic effect of parathyroid is, therefore, not dependent on its renal phosphate effects.

In recent extension of these observations, Talmage & Elliott (72) have studied changes in extracellular fluid levels of calcium, phosphate, and citrate ions in the nephrectomized-parathyroidectomized rat using Grollman's peritoneal dialysis technique. They attempted to relate the extra-renal effects of parathyroid hormone on calcium and phosphate to the renal effects, both as to the time relations and the manner in which the two ions are affected. They showed that the extra-renal actions of parathyroid hormone occur in just as short a time as the renal ones and that the changes in extracellular concentrations of calcium, phosphate, and citrate ions are all of approximately equal magnitude. These studies support the thesis that the parathyroids maintain a constant plasma level of calcium by making available calcium and phosphorus from bone while simultaneously raising the renal threshold for calcium and lowering that for phosphate. The citric acid elevation in blood following nephrectomy has been shown to be dependent on intact parathyroid glands (73). Whether the hypercitricemia and hypercalcemia in response to parathyroid extract are both due to a direct action of the hormone on bone or whether partly due to a renal effect of parathyroid on citric acid excretion is not clear from these studies. Further research is certainly needed to unravel still more completely these complicated interrelationships between calcium, phosphate, and citrate and their control by the parathyroid.

GLUCOSE TRANSPORT

Chinard and his associates have recently applied their elegant techniques for studying rate of excretion of glomerular and nonglomerular substances to an *in vivo* analysis of some aspects of glucose reabsorption by the dog kidney (74). A solution containing T-1824, glucose-1-C¹⁴ and creatinine was injected nearly instantaneously into the renal artery. Thirty successive samples of renal venous blood were rapidly collected at 1.4 sec. intervals and the concentration of injected substances in each blood sample was determined and divided by its concentration in the injected solution. These resultant concentration ratios were then plotted against sample numbers (hence time). By radioactive studies, degradation, and chromatographic techniques it was conclusively shown that glucose traverses the renal tubule cell unchanged, without any breakdown and resynthesis of the six carbon chain. This has also been shown by one of the authors to be the case with glucose transport across the intestine (75). Simultaneous curves of glucose and creatinine appearance rates in renal venous blood allowed the authors to measure "mean transit time" for the glucose from injected site, through glomeruli,

across tubules to renal vein catheter tip. The mean time thus calculated for glucose in eight experiments was 10 sec. If it be assumed that the mean transit time for glucose from glomerular barrier to luminal border is 5 sec., then the transit time of glucose across the tubular cells is also of the order of 5 sec. These calculations, when combined with estimates of tubule cell thickness and cytoplasmic viscosity, led the authors to the conclusion that the glucose carrier complex is not a macromolecule and that the transport does not directly involve organized intracellular particles such as mitochondria. After the injection of phlorizin the excretion patterns of glucose and creatinine were identical, and from the theoretical transit time considerations just outlined their inference was strengthened that the combination of glucose with the "carrier" occurs at the cell barrier rather than within the cell. This supposition is in harmony with the thinking of Krane & Crane (37) and Rosenberg & Wilbrandt who favor a surface carrier for glucose much more than one inside the cytoplasm (42).

This new and dynamic approach to active transport problems offers a beautiful opportunity to study carefully the *in vivo* kinetics of these mechanisms. Of course one is dealing with a multiunit system and thus one must be cautious when drawing far reaching conclusions about kinetics of a "carrier." It would be of great interest to see the effects of carefully graded doses of phlorizin on the mean transit time of glucose in the kidney using the Chinard technique. Such studies would perhaps provide a firmer base for a description of the position of the carrier than is provided by the above discussed study in which rather far-reaching conclusions are drawn (albeit speculatively) from as yet limited data.

SOME INHIBITORS OF TRANSPORT

Phlorizin.—Consideration of the mechanism of glucose transport in the kidney leads quite naturally to a consideration of the glycoside phlorizin, which has long been known for its capacity to inhibit glucose reabsorption. Some recent work on the mechanism of action of phlorizin deserves consideration. To block such a mechanism as glucose reabsorption, phlorizin would have to (a) compete directly with glucose for the system, (b) inhibit a cell surface carrier by attaching to it, or (c) gain entrance to the cell and inhibit either a cytoplasmic carrier or the energy-producing reactions of the cell that drive the active transport of glucose. It is hard to conceive of a competitive inhibition of the transport system because phlorizin is effective in such extremely small doses. Unpublished experiments in the author's laboratory³ show that T_m of glucose in the dog is inhibited 50 to 60 per cent by as little phlorizin as 2 μ g per kilo per min. In our experiments this represented a total cumulative dose over seventy minutes of only 2.8 mg. Thus the glucose transport system is remarkably sensitive to this inhibitor and these amounts could hardly represent competitive inhibition between glucose

³ This work is being supported by a grant from the Life Insurance Medical Research Fund of New York.

and phlorizin in the sense of succinic and malonic acids, for instance. The work quoted earlier of Krane & Crane (37) on effect of phlorizin on D-galactose 1-C¹⁴ uptake in kidney cortex slices and of Chinard *et al.* (74) on glucose absorption in the dog are both consistent with a surface carrier binding action of phlorizin or one that alters the diffusibility of glucose into the cell.

Rosenberg & Wilbrandt (42) have studied the relative potency of phloretin and ten related compounds as inhibitors of glucose transport across the human erythrocyte membrane. The hydroxyl groups of one of the phenol rings of phloretin (the aglucone of phlorizin) were substituted by OCH₃ or glucose. Phloretin itself is the most potent inhibitor of glucose transport across the erythrocyte membrane (more so than phlorizin which is the more potent glycosuric of the two in animals). Substitution of the OH groups was found to diminish the inhibitory effect of the phloretin, particularly if the substitution is at the 4 position. 2,6-Dimethylphloretin is quite inactive as an inhibitor. The authors show that the phloretin molecule with ring closure closely resembles the steroids, some of which, particularly deoxycorticosterone, are known to be glycosuric in animals. The authors theorize that such ring closure, through hydrogen bonding or metal chelation with the carrier complex on the cell surface, binds the surface carrier which transports glucose and thus inhibits the mechanism. To substitute these hydroxyl groups with one that prevents such ring closure robs the phloretin molecule of its inhibitory properties. This is an ingenious idea which Rosenberg & Wilbrandt support by showing that deoxycorticosterone or its glycoside are themselves inhibitors of glucose transport across the red cell membrane. Thus it is perhaps in a closed, steroid-like form that phlorizin and phloretin are effective glucose transport inhibitors and perhaps it is at a surface carrier site that they work.

Shapiro (76) suggested that phlorizin has its effect by interfering with both glycolysis and aerobic oxidation in kidney and reducing the capacity of the cells to generate high energy phosphate bonds. Thus there would be impaired energy supply for the glucose transport. Lotspeich & Keller (77) have continued to study the effect of phlorizin on energy metabolism in kidney. Whereas Shapiro had used a mince of rat kidney, these workers used washed homogenates of guinea pig kidney fortified with magnesium ions, ATP and a single substrate. They found that phlorizin in small concentrations (10^{-3} to 10^{-4} M) inhibits the oxidation of any of the substrates of the tricarboxylic acid cycle and others (glutamate or β -hydroxybutyrate) which are oxidized through the electron transport chain. They further found that an excess of either ATP or ADP or AMP would partially or completely prevent the phlorizin block. They postulated that phlorizin inhibits the transfer of inorganic phosphate to ADP during the coupling between oxidation and phosphorylation in the terminal events of tissue respiration in the electron carrier chain leading from substrate to molecular oxygen. These same workers have extended their observations to mitochondria from rabbit kid-

ney and liver (78) where α -ketoglutarate is substrate and the coupling between oxidation and phosphorylation is good (P/O ratios 2.5 to 3.0). These studies have substantiated the earlier ones, namely that phlorizin inhibits oxidation in the electron carrier chain by a loose, probably noncompetitive type inhibition.

There is no doubt that phlorizin affects tissue oxidation *in vitro* (76, 77, 78) and that it does so in reasonably small concentrations. There is also no doubt that it has been shown to affect glucose and galactose transport *in vitro*, in a similar concentration range, in kidney slices, Ehrlich ascites tumor cells, and in erythrocytes in a way unrelated to oxidative metabolism and suggesting a surface carrier or diffusion interference. One wonders how the two disparate observations are related. One possible relation comes to mind. It is conceivable that the oxidative metabolism of the cell is essential for the maintenance of a surface carrier. This would be the case where ATP and ADP are involved in phosphate transport across the cell wall of *Staphylococcus aureus* as visualized by Mitchell (22). Furthermore the maintenance of the integrity of the cell wall itself as a functioning entity has been shown to depend upon an intact cell metabolism. Park & Strominger (79) have shown that penicillin inhibits the capacity of the coenzyme uridine diphosphate glucose to function in its essential role of synthesizing the amino sugars which form an integral part of the cell wall in certain bacteria. Hence in the presence of penicillin the cell wall breaks down as the forces of catabolism overcome those of anabolism and the organism becomes disrupted as can be seen under the microscope. It is not inconceivable (as we have discussed above in the case of phosphate) that the cell surface carrier is something like a uridine phosphate coenzyme which would transport glucose across the cell membrane as uridine diphosphate glucose and thus not alter its chain configuration [as has been shown to be the case (74)]. On the other hand, such coenzymes might be involved in the maintenance of the structural integrity of the tubular cell wall, and since these coenzymes themselves depend on cell metabolism, a disruption there would affect them in turn. The speed with which phlorizin works and its conspicuous lack of any microscopically recognizable cellular-damaging effect would speak against such a membrane-altering effect as that of penicillin. Any effect such as that would have to be ultramicroscopic, indeed. At the moment, therefore, we have conflicting *in vitro* effects of phlorizin before us without any other more sensible synthesizing interpretations apparent than those just discussed. Perhaps the "gentle reader" will be sufficiently enlightened to illuminate this darkness.

Mercurials.—Important studies have appeared representing continued attempts to understand the nature of mercurial diuresis. One group have studied extensively the distribution and fate of isotopically labelled chlormerodrin in the dog and rat and have attempted to correlate diuresis with renal cortical concentration and excretion of this compound (80, 81). In an extension of their experiments, Kessler, Lozano & Pitts (82) have attempted

to correlate structure of mercurial compounds with diuretic activity in dogs. Twelve organic mercurials and mercuric chloride were studied in dogs. Diuresis and natriuresis were used as indices of "diuretic activity." Following the experiments the kidneys and several other organs were removed and their mercury concentrations determined. A Geiger counter over the exposed kidney recorded the uptake of mercury in the outer few mm. of renal cortex during the experiment. The study sprang from the present confusion about the mechanism of action of the mercurials. Their diuretic activity has been ascribed to their inhibition of sulfhydryl enzymes associated with sodium and chloride reabsorption (83). There has also been considerable discussion of whether the active diuretic form of mercury is organic or inorganic. To help clarify their problem Kessler and his colleagues asked themselves three questions: (a) Is the carbon side chain of an organic mercurial essential for its diuretic action? (b) Are sulfhydryl inhibitors necessarily diuretics? (c) Can diuretic activity of a mercurial be related in any way to its distribution and fate in the body? Their research growing out of these questions led them to the conclusion that there is no particular relation between diuretic activity of a mercurial and its rate of excretion in the urine, intensity of concentration in the kidney, or its action as a sulfhydryl enzyme inhibitor. Their results could only be explained by postulating that a critical steric configuration of the mercurial molecule is necessary to allow it to fit into a "lock and key" relationship with some essential component of the sodium and/or chloride reabsorptive mechanisms. This is a molecule with a chain of not more than three carbon atoms, an atom of mercury with an open valence attached to the terminal carbon, and some hydrophilic group not less than three carbons distant from the mercury.

These mercury compounds are all more or less completely bound to plasma proteins; therefore, they must be excreted into the urine by the tubules. Indeed, direct evidence for this has been obtained in the chicken (84), adding weight to the indirect evidence supporting this conclusion for dogs and rats.

Müller & Weiner (85) have studied the excretory products of the organic mercurial diuretic mersalyl and have shown that this mercurial is excreted as a thiol complex similar to that previously demonstrated by them for chlormerodrin. With mersalyl there are two mercury thiol compounds demonstrable polarographically in the urine. Thiol I is probably a cysteine complex formed in the kidney (and secreted into the urine by the tubules); Thiol II, an N-acetyl cysteine complex of the mercurial formed in the liver and brought to the kidney. Are these thiol mercury complexes the active diuretic form? Kessler, Lozano & Pitts (82) raised this question in their paper and attempted to reconcile this possibility with their idea of a critical steric configuration for diuretic action. They showed that in a cysteine complex, if sulfur could replace carbon, the chain of one sulfur and two carbons between mercury and the hydrophilic amino group would constitute a configuration very close to that they consider essential from their studies. How-

ever, evidence has been presented supporting the idea that the thiol form of mercury is not necessarily the active one. Weiner, Levy & Mudge (86) have found thiol excretory complexes not only of the diuretic mercurials, meralluride, and mercuric chloride, but of the nondiuretic compound, *p*-chloromercuribenzoate, as well. Perhaps this dilemma will be shown to be peripheral to the main question. Maybe thiol complexing, which occurs both in kidney and liver with a host of diuretic and nondiuretic mercurials, is not really related to the central problem of the diuretically active molecule and its site of action.

The assumption is made in all these studies that it is organic mercury rather than the inorganic that is the effective form for diuresis. Studying the effects of acid-base balance on the diuresis induced by both organic and inorganic mercurials, Levy, Weiner & Mudge (87) have thrown doubt on this idea and present rather striking evidence to favor inorganic mercury as the active form.

Like the preceding discussion of phlorizin, this mercurial diuretic problem is at the moment clouded with intriguing but conflicting observations. Perhaps more studies are needed with model systems such as yeast, the erythrocyte or the rat diaphragm where, working with an isolated more easily controlled system, one can study the effect of a wide variety of mercurials (organic, inorganic, and thiol complexed) on sodium and chloride fluxes *in vitro*. We need done for the mercurials what has been done with a variety of other inhibitors in order to identify the exact mercury-sensitive sodium and/or chloride carrier system. Then knowing this, we can return more intelligently to the whole animal.

ACID-BASE REGULATION

SYNTHESIS AND EXCRETION OF AMMONIA

The mechanism of synthesis of ammonia has continued to interest a number of investigators. This has particularly centered on the glutaminase enzymes in kidney and their role in the ammonia mechanism. The induced adaptive increase in renal glutaminase observed by Davies & Yudkin (88) has stimulated further studies of this phenomenon. Goldstein, Richterich-van Baerle, & Dearborn (89) have observed glutaminase hypertrophy in guinea pig kidney following prolonged administration of either acid or alkali, a surprising finding which, however, is consistent with their earlier observation that ammonia excretion is high in urine of pH 6, drops down at pH 7.5 and then goes up again to increased levels at pH 8.5 (90). These authors have studied three separate enzymes involved in glutamine metabolism: glutaminase I, a hydrolyzing enzyme with pH optimum of 7.4; glutaminase II, also a hydrolyzing enzyme but with a pH optimum at 8.8 and dependent on the presence of alpha-ketoglutarate, an enzyme similar to the glutamine transaminase-deamidase of liver described by Meister (91); and finally, a glutamine synthesizing system which forms glutamine from glutamic acid and ammonia. The rats were given NH_4Cl or NaHCO_3 chronically and then

kidneys assayed for these enzymes. In acidosis only glutaminase I activity was increased, while in alkalosis all three enzymes showed elevated activity. These findings certainly represent the addition of important new knowledge in this field. One wonders about the relation of this finding to the observation of elevated ammonia excretion in alkalosis as well as acidosis (90) and how this latter observation is explained in the light of Orloff & Berliner's observations, and careful analysis, of a consistently falling ammonia excretion in alkaline urines (44). Orloff & Berliner worked with dogs and did not reach urine pH's as high as those in the present study with guinea pigs (90). Nevertheless the physicochemical limitations upon ammonia diffusion out of the cell at very alkaline pH discussed by Orloff & Berliner still operates and should not be too heavily conditioned by species difference.

Richterich-van Baerle and Goldstein have presented a limited analysis of the kinetics of renal glutaminase increase in acidosis (92) in an attempt to use this phenomenon to study induction of an enzyme in mammalian tissue, much as Knox has studied tryptophane peroxidase in liver (93). Although an interesting paper, it is hoped that the authors will study the glutaminase induction phenomenon more exhaustively and present a more detailed work at a later date. Certainly the glutaminase induction phenomenon provides a beautiful opportunity to carry into higher forms the sort of induction studies done so elegantly in microorganisms (94, 95).

Seldin, Teng & Rector (96) have made an interesting comparison of changes in excretion of ammonia and titratable acid and level of renal glutaminase in rats given strong acid (ammonium chloride) or buffer acid (sodium acid phosphate). These investigators were interested to learn whether the increased renal glutaminase results from "pre-renal or renal effects of an acid load." In the rats given NH_4Cl there was increased ammonia excretion and glutaminase activity with very little change in titratable acid excretion. On the other hand, in the rats given an equivalent load of buffer acid there was very little increase in ammonia, no change in glutaminase, but a large increase in titratable acid. Thus, they conclude, the glutaminase increase which results from a strong acid rather than a buffer acid load is caused by unidentified changes within the kidney rather than something before the kidney. Although this conclusion is what one might expect, the clear-cut differences in ammonia and titratable acid excretion with the two different but equivalent acid loads were striking. This experiment would make an elegant project exercise for a physiology laboratory class.

Wilson & Seldin (97) have restudied the effect of adrenalectomy on the production and excretion of ammonia by the kidneys. Ever since Wilhelmi showed that kidney slices from adrenalectomized rats synthesized less ammonia than controls (98) this problem has interested renal physiologists. Sartorius, Calhoun & Pitts (99) and Harris, *et al.* (100) have observed decreased capacity to produce renal ammonia in the adrenalectomized rat and dog. Particularly in the rat it is, indeed, precarious to give a strong acid load to the adrenalectomized animal; cardiovascular collapse is easily produced.

This fact has been reobserved by Wilson & Seldin (97) who found that the survival of such rats on ammonium chloride could be prolonged by restricting potassium intake to reduce the hyperkalemia of adrenal insufficiency. Certainly these are the best experiments yet presented in this difficult to control problem; these authors find a decreased ammonia-producing capacity as a result of adrenalectomy. Oddly enough the usual adaptive increase in glutaminase occurred despite adrenalectomy. Could it be that the adrenal role in ammonia excretion is realized in the diffusion of the substance into the lumen rather than its synthesis in the cell? Or perhaps the hydrogen ion secretion is at fault and NH_3^+ is not adequately converted to NH_4 . Experiments on the effect of adrenalectomy on renal ammonia production will not be completely unequivocal and acceptable until side changes in filtration rate and renal blood flow are controlled. Neither Sartorius *et al.* (99) nor the present authors have presented blood flow and filtration rate data in their papers. This seems a serious omission, for the reviewer recalls sharp reductions in creatinine clearance in adrenalectomized rats given ammonium chloride in his own laboratory.

The exact tubular site of addition of ammonia to the urine is still a mystery. Walker (101) observed in the frog that it started to appear in his tubular micropuncture samples about the middle of the distal tubule and reached highest concentration in the collecting ducts. Recently Richterich-van Baerle, Goldstein & Dearborn (102) have reached a similar conclusion from a different approach. They have noted a correlation between ammonia production and glutaminase I activity in tissue (as noted above). Whereas glutaminase I was observed throughout the kidney, most of its activity was either in cortex or papilla. This is consistent with distal tubule and collecting ducts as chief sites of ammonia formation.

The exact way in which glutamine and the glutaminases function in ammonia production remains obscure, as does the meaning of the glutaminase increase phenomenon. Certainly glutamine (103) and certain amino acids (104) act as substrates for urinary ammonia, but the intermediary steps of these processes are not at all clear. Perhaps one should try to identify the end products of the reactions of ammonia synthesis *in vivo* in acidotic dogs infused with either glutamine or a single amino acid. If administered in fairly large amounts, the reaction products should appear in detectable amounts in the renal vein blood. Certainly, with respect to the amino acid substrates, transamination is more likely to be a critical reaction than straight deamination, particularly in view of the small amounts of L-amino acid oxidase in kidney. If the amino acid is undergoing transamination to any large extent during ammonia synthesis, one might expect to see new keto acid and new amino acid in renal vein; if, on the other hand, deamination were the main reaction, the keto acid corresponding to the amino acid infused might be found coming from the kidney. Perhaps glutamine and glutaminase are not really the critical components of the renal ammonia mechanism; perhaps they are like carbonic anhydrase-carbonic acid in gastric acid secretion, involved but not rate limiting (105). Perhaps the glutamine transaminase-

transamidase system operates with glutamine to maintain a glutamic-alpha ketoglutarate transaminase system or glutamic acid-glutamic dehydrogenase are critical and glutamine-glutaminase somehow is maintaining this system intact. Although these are only possible alternatives, they are posed lest we become so intrigued with glutamine and glutaminase that we fail to even question the essential nature of their role.

CARBONIC ACID, BICARBONATE, AND CARBONIC ANHYDRASE

Coulson & Hernandez (106) showed that the alligator kidney produces ammonia for cation conservation and carbonic acid for anion conservation. This carbonic acid secretion involves carbonic anhydrase. Thus when one gives Diamox to the alligator, there is a decrease in urinary bicarbonate rather than an increase. This decreased bicarbonate excretion after Diamox is accompanied by an increased loss of chloride. In an extension of these studies (107) the same authors saw what happened to this Diamox chloruresis when plasma level of chloride or the other anions, sulfate or phosphate, was artificially elevated. With choride administration the familiar Diamox chloruresis was simply magnified. The administration of sulfate or phosphate, on the other hand, depressed chloride excretion to such an extent that when Diamox was given the usual chloruresis did not occur. In this animal where carbonic acid secretion is the counterpart of hydrogen ion secretion in other forms, one wonders how NH_3 is converted to NH_4 or conversely how ammonia gets into the tubular urine if normally this "hydrogen trapping" of NH_3 is critical for establishing a diffusion gradient for ammonia across the tubule border. These curious reverse relationships in the alligator provide an intriguing example of an adaptation of a fundamental mechanism to meet the particular environmental needs of one animal.

These same investigators have also studied the effect of cold on the tubular function of the alligator and a small group of turtles (108). As water temperature is progressively lowered from 28°C. to 1°C., tubular function is steadily reduced until the urine comes to resemble glomerular filtrate. This same phenomenon was noted in frogs by Hong (109).

The diminishing effect of repeated doses of Diamox has received attention from two groups of workers: Hanley & Platts in England (110) and Maren in this country (111). The loss of bicarbonate following Diamox administration lowers plasma bicarbonate which itself changes the filtered load and the tubular reabsorptive capacity of this ion. These changes invoke an automatically self-continuing mechanism where a new level between filtered and reabsorbed bicarbonate is reached and here the inhibitor is without effect. Since the clinical use of Diamox depends on its ability to reduce base-bicarbonate reabsorption, this "resistance" phenomenon seriously limits its clinical usefulness where base loss is desired.

ACIDOSIS AND ALKALOSIS

Fuller and MacLeod (112) studied changes in arterial PCO_2 , titratable acid and ammonia excretion in dogs during acute respiratory acidosis (in-

halation of CO_2) and respiratory alkalosis (hyperventilation). In the acidosis there was a rise in both free acid and ammonia excretion which returned to near control values when the CO_2 inhalation was stopped. On the contrary, in respiratory alkalosis, although free acid and ammonia excretion fell, as one would expect, these parameters did not return to control levels during the recovery periods but remained depressed for some time. Although pointed out, these changes are not explained or their significance assessed by the authors.

Barker, Singer & Elkinton (113) have presented an analysis of the renal response in man to acute respiratory alkalosis and acidosis. The reader is referred to this paper and to Elkinton's excellent review (7, 8) for a complete review of this subject in which this group has made such fundamental contributions.

WATER, ELECTROLYTES AND OSMOREGULATION

POTASSIUM

Since it was first demonstrated that potassium is secreted by the tubules (114, 115) as well as reabsorbed, the relative roles of these two processes in the overall excretory process and general homeostasis of this ion has not been clear. Several recent studies, however, have helped our understanding. Koch, Brazeau & Gilman (116) found that under conditions of normal or below normal potassium levels in the body, sodium and potassium in the glomerular filtrate are reabsorbed as cation species indistinguishable from one another in relation to an active chloride reabsorption which "drags" these two unidentifiable species after it. However, when there is an excess of potassium in the body, as in high potassium diet, potassium secretion comes into play and net tubular flux is on this side. The data also indicate that potassium secretion involves an exchange process with sodium reabsorption (117, 118). Thus the activity of the secretory process appears to depend on the simultaneous availability of both participants in the exchange reactions. The authors conclude that these reactions occur in the distal tubule. These findings are in close harmony with results from experiments on the artificially perfused isolated frog kidney (118) and the studies of Morel & Guinnebault (119) who injected K^{42} into rabbits and followed the sites of its deposition in the several zones of the kidney by measuring specific activity of the tissue and studying radio-autographs. Another group of workers (120) have also come to the conclusion that potassium is secreted during its infusion to elevate plasma levels. The effect in humans of extracellular pH change on the relation between serum potassium and intracellular potassium concentration has been studied (121). Changes in extracellular pH alter extracellular potassium independently of changes in total body potassium. Thus in animals acidosis increases and alkalosis decreases serum potassium independently of total body potassium. An analysis of a relationship between hydrogen ion and potassium ion in the active transport of sodium across isolated frog skin (122) may have an explanatory bearing on these and similar observations.

The following references should also be consulted for those probing more deeply into the relation between pH and cation movement and the interrelations between sodium, potassium and other cations across cells (123 to 128).

INORGANIC SULFATE

Previous experiments had shown that inorganic sulfate is freely filterable and exhibits a reabsorptive T_m (129). Furthermore this T_m is reduced by chloride (130) or several amino acids (131). Subsequently it was discovered that sulfate reabsorptive rate, along with those of acetoacetate and inorganic phosphate, is depressed by glucose and increased when phlorizin is administered (132). Deyrup, using kidney cortex slices *in vitro*, has measured the uptake of inorganic sulfate labelled with S^{35} under a variety of conditions (133) and has found that many of the conditions affecting sulfate transport in the kidney of the dog *in vivo* also affect its uptake in slices *in vitro*. Thus chloride depresses and phlorizin (as *in vivo*) strikingly increases $S^{35}O_4$ uptake. Deyrup feels that she can correlate conditions of transport rather well in her *in vitro* system with what happens *in vivo*. Berglund & Deyrup (134), using a chemical technique for measuring sulfate, have confirmed Deyrup's original observations on sulfate uptake where she measured the isotope S^{35} .

Apparently stimulated by the relationship between sulfate, phosphate and acetoacetate transport on the one hand and glucose and phlorizin on the other (132), a group of workers in Berlin (135) have looked for a correlation between tubular transport mechanisms for glucose and some monovalent cations, reasoning that if there is such a relationship between glucose and several anions, perhaps cations are related as well. They used the isolated artificially perfused kidney of the frog, *Rana esculenta*, where renal portal and arterial systems of the kidney can be separately perfused. Measuring potassium and sodium fluxes across the perfused tubules in the absence and presence of glucose and phlorizin, they found that these substances in no way altered K^+ or Na^+ transport and thus concluded that there was no relationship between glucose and these two monovalent cations in tubular transport, whatever may be the interrelations with the anions previously studied (132). One wishes that they would study the "anion-glucose-phlorizin" relation also in their frog preparation.

WATER AND OSMOREGULATION

Antidiuretic hormone (ADH), concentrating and diluting mechanisms.—Wirz has continued his elegant experiments on the renal concentrating and diluting mechanism with a new study of the osmotic pressure in the cortical tubules of the rat kidney (136). In anesthetized rats the several parts of the tubule, excluding the loop of Henle, were entered by micropuncture. Samples of urine were withdrawn and freezing point determined by a microkryoscopic technique during conditions of water loading (the "diluting kidney") and dehydration (the "concentrating kidney"). He has confirmed the observation that proximal tubular urine is isosmotic with plasma during normal hydration and water diuresis where ureteral urine osmotic pressure is well

below that of plasma. The contents of the first part of the distal tubule are definitely hypotonic both during water loading and when kidneys are concentrating. Where kidneys are making a dilute urine this hypotonicity is maintained throughout the entire length of the distal segment. On the other hand, when concentrated urine is being formed the hypotonicity gives way to isotonicity from the middle of the distal convolution onwards. Wirz reasons that the fluid becomes hypotonic before it enters the distal convolution by the reabsorption of solutes (Na^+Cl^-) through a membrane which is virtually impermeable to water (*Wasserundurchlässig*) in both the diuretic and anti-diuretic state. ADH is visualized by Wirz as operating in the distal tubules where it "opens pores and thus makes a water permeable out of a water impermeable membrane." He compares this action of ADH with a similar effect on frog skin as studied by Koefoed-Johnsen & Ussing (137) and rules out any active transport of water here in the distal tubule, ADH only creating a permeability to allow for the passive transfer of water. The final concentration of the urine takes place in the collecting ducts by the operation of the "hairpin countercurrent" mechanism originally proposed by Hargitay & Kuhn (138).

The observed facts presented in this work are sound and represent a triumph of method for which Wirz is to be congratulated. Certainly it appears that ADH changes the permeability of the distal tubular epithelium to water; whether the analogy with the frog's skin is warranted is not apparent until more evidence of the effect of ADH on the water permeability of a number of cell surfaces is before us. Although the countercurrent hairpin mechanism of urinary concentration has many questionable aspects, it offers an ingenious working hypothesis which is the way Wirz and his colleagues apparently regard it at the moment.

Continuing earlier studies of theirs where they observed that conditions producing engorgement of the thoracic viscera produce diuresis (139), several of the Wright Air Development group have sought further evidence that urine flow is at least partially controlled by thoracic volume receptors (140). In order to separate volume receptor effects from osmoreceptor response, these investigators infused into dogs isosmotic volumes of human serum albumin, saline, and whole blood. Whereas none of these procedures would be expected to change plasma osmotic pressure, in each case urine volume was significantly increased. The authors conclude that, although ADH concentration of donor blood may alter the diuretic response, their experiments support the concept of volume receptors for urine flow. These same investigators in a later paper (141) also present experiments in which intracarotid injections of hyperosmolar solutions caused antidiuresis and thus additionally support Verney's concept of a diencephalic center sensitive to the osmotic pressure of the blood perfusing it (142). Presumably both "volume receptor" afferents (over vagus?) and osmoreceptor afferents (over supraopticohypophyseal tract) effect release of ADH in the neurohypophysis. The afferent limb and central connections of the volume receptor mechanism have not yet been worked out in detail; an elucidation of these facets

of the "reflex" will form the completion of a neat story in contemporary physiology. A similar volume-sensitive mechanism has been postulated for aldosterone release and will be discussed below.

A method has been presented for assaying ADH in plasma (143) and showing that ADH is completely absent from blood of diabetes insipidus dogs maintained for as long as a year. Two groups of investigators have studied the effects of the vasopressin and oxytocin principles of posterior pituitary on water and electrolyte excretion in the rat (144, 145). An oxytocin preparation produced an increase in water, sodium, and chloride excretion during 120 min. following its administration. This effect disappeared along with uterine action after chymotrypsin treatment (144). A vasopressin preparation was antidiuretic and antinatriuretic, and these properties remained even after chymotrypsin had destroyed coincident oxytocic activity. A synthetic oxytocin preparation was shown by Swiss workers (145) to have a bivalent effect on diuresis: In high doses it is antidiuretic in the sense that the interval between oral water load and maximum diuresis is prolonged (probably side renal vascular effects?); it is diuretic in the sense that total urine volume during the period of enhanced diuresis is increased. These studies on oxytocin and vasopressin are important since the brilliant discovery of their structural formulae (146) and the synthesis of oxytocin from amino acids (147), both pieces of work from Du Vigneaud's laboratory.

Finally there has appeared a paper by Kleeman, Epstein & White (148) reporting observations on the effect of variations in solute excretion and glomerular filtration rate on water diuresis. Their results indicate that, during water loading, both glomerular filtration rate and solute load markedly affect the water diuresis, the free water clearance being not constant, as it is during the hydropenic osmotic diuresis seen in Wesson & Anslow's experiments (149).

ADH effect on water and electrolyte excretion.—Wrong (150), studying the relation between water retention and salt diuresis following vasopressin, feels that the chloruresis and natriuresis that results is a function of the accompanying fluid retention, a physiologic response to over-expanded extracellular fluid. He gave water rapidly along with vasopressin to three male subjects and collected urine at frequent intervals. A marked natriuresis (with bicarbonate as anion) appeared 10 to 14 hr. following this over-hydration procedure. Wrong suggests that the delay in electrolyte diuresis is the result of slow disappearance of aldosterone from the circulation. That there is a suppression of aldosterone secretion under such conditions of expanded extracellular fluid volume has, indeed, been shown [see discussion below of Bartter's work (1957)]. Whether the disappearance of aldosterone is "slow" one cannot say from available data. Laragh & Stoerk (151) have shown that aldosterone in urine increases as serum potassium is elevated. If Wrong's delay in natriuresis were the result of continuing presence of aldosterone in the circulation, one would expect to see natriuresis coincide with a fall in serum potassium which would suppress aldosterone release from the adrenal (according to Laragh's hypothesis) and allow natriuresis to occur.

Wrong does not discuss serum potassium levels in his over-hydrated subjects nor does he record urinary aldosterone excretion. Certainly these parameters would be helpful in an overall interpretation of his interesting results.

ELECTROLYTE EXCRETION AND REGULATION

Aldosterone and interrelations with ADH in control of electrolyte excretion.—

Since its isolation and initial characterization as a potent sodium-retaining adrenal cortical hormone (152), aldosterone has received much attention. Conn's now classic description of primary aldosteronism (153) has furnished a background against which research on this hormone rests, just as Addison's first description of clinical adrenal insufficiency clearly delineated the lines of later work. Recent papers, accordingly, have sought an explanation of (a) the mechanism whereby aldosterone affects sodium reabsorption in the kidney, (b) the nature and storage place of a trophic hormone for aldosterone, and (c) the mechanism whereby aldosterone is released.

Dustan, Corcoran & Page (154) have studied renal function in three patients with primary aldosteronism before and after removal of adrenal tumors. They showed typical sodium retention with excessive potassium excretion, primary effects of aldosterone operating on the renal tubule which has interrelated Na^+ and K^+ transport mechanisms (116, 117, 118). Polyuria and excretion of dilute urine are sequelae of the resulting potassium deficiency. In the patients with aldosteronism there is nothing wrong with acidification or ammonia synthesizing systems; why then is the urine in this condition characteristically alkaline? The evidence at hand indicates that intracellular potassium is the determining factor in urine pH, excretion of Na^+ and K^+ and the ratio of basic to hydrogen ion in urine (155, 156). Aldosteronism seems to violate this evidence; there is alkaline urine while tubular cells have low potassium and high PCO_2 . Dustan, Corcoran & Page resolve this conflict by postulating that aldosterone favors the ionization of potassium within the tubular cell. Thus even though the cell is K^+ -depleted, the excess aldosterone accelerates K^+ secretion.

In these studies one could also assume that aldosterone alters the permeability of the cell to K^+ . A study of the effect of aldosterone on K^+ transport in an *in vitro* preparation and its effect on the volume of distribution of K^{42} *in vivo* in the nephrectomized animal would be most useful at this point.

The regulation of aldosterone release has received much experimental attention. Bartter and his associates (157) were able to confirm the fact that urinary aldosterone-like steroids are increased during sodium restriction (158) and extended their studies in an attempt to clarify the relation between sodium balance and aldosterone secretion. In a series of ingenious studies on human subjects extracellular fluid volume and tonicity were altered coincident with and independent of intracellular fluid volume and tonicity. In all their experiments changes in urinary aldosterone (and presumably in aldosterone secretion from the adrenal) followed directly reciprocal changes in extracellular fluid volume when this volume was varied either with or with-

out similar changes in sodium. Thus these authors are convinced that extracellular fluid volume (and not intra- or extracellular ion or water concentration) is the stimulus to aldosterone release and that changes in serum sodium per se are unlikely to stimulate aldosterone secretion. Laragh & Stoerk (151) came to the conclusion that increased serum potassium is the physiologic trigger releasing aldosterone. Bartter and associates (157) discuss this disparity in findings but offer no explanation. They suggest that the normal control of water and sodium balance depends on a double "feed-back" mechanism involving kidneys, adrenals, and the supraopticohypophyseal system. Aldosterone, by virtue of its ability to increase sodium reabsorption in the kidney, causes sodium retention. This results in a rise in extracellular fluid volume. Some function of this increased volume then serves in turn to inhibit aldosterone secretion. Although the authors have no evidence revealing how extracellular fluid volume affects adrenal cortical secretion, one wonders whether it might not involve the same intrathoracic volume receptors which have been shown to affect urine volume (139).

Cole, in a series of imaginative papers (159, 160, 161), has presented a theoretical account of a possible interaction of antidiuretic and sodium-retaining hormones in the kidney where he has considered a model of the tubules based on properties of a membrane. His membrane properties are deduced from Ussing's work with the frog skin. In such a system ADH would increase the permeability of the membrane to water, sodium reabsorption being an active process under aldosterone control. These studies represent an ingenious and useful presentation based largely on transpecies and trans-tissue extrapolations as well as highly theoretical mathematical deductions. Cole has also presented a study showing an increased retention of intravenously administered saline in rats (162). As far as the reviewer knows this is the first study showing such a direct effect; others having shown it indirectly. Papers have also appeared presenting cases of primary aldosteronism without any recognizable morphological changes in the adrenal cortex (163) and increased aldosterone activity in urine during chronic hemorrhage in dogs (164).

The evidence for believing that aldosterone secretion is independent of anterior pituitary control has been well summarized by Laragh & Stoerk in the introduction to their paper (151). Now Rauschkold & Farrell have evidence for believing in an extrahypophyseal diencephalic center regulating aldosterone secretion (165). These authors collected adrenal venous blood in dogs which had been either decapitated, decorticated, decerebrated, or had had spinal transection. The samples of adrenal vein blood were assayed directly for aldosterone. They found a marked reduction in rate of aldosterone secretion in both decapitate and decerebrate preparations indicating to them that the center for controlling aldosterone release must reside in the brain, probably in the diencephalon. One wonders why these investigators failed to report experiments on the hypophysectomized dog; it would have made the results so much neater. Furthermore, one is worried by the fact that in those very preparations where aldosterone secretion was reduced, the

secretion of hydrocortisone was reduced also. Hydrocortisone itself is the steroid most actively released from the adrenal in response to pituitary ACTH injection; therefore, on this count also this paper, which represents such an elegant approach to this problem, falls short of a complete and decisive experimental demonstration.

Certainly we should see experiments done where various thalamic and hypothalamic areas are both stimulated and discreetly destroyed with the stereotaxic instrument in order to see the effects of these maneuvers on aldosterone secretion from the adrenal using the Rauschkold & Farrell technique (165). This technique would also be useful in attempting to resolve the mechanisms whereby extracellular fluid volume, sodium or potassium (or all together) stimulate the brain mechanism to signal adrenal release of aldosterone. What would happen to adrenal vein aldosterone when extracellular fluid volume is expanded and then the vagi cut? What would be wrong with perfusing the isolated adrenal with solutions of differing tonicity and composition to study the effects of these variables on the aldosterone in the gland effluent?

BODY SPACES, ION AND WATER MOVEMENTS

The discussion continues over whether intracellular water is regulated actively by a "water pump" or whether the primary mechanism is an "ion pump" with water following passively. Robinson (166) studied the relation between water content of kidney cortex slices and their respiration rate under a variety of circumstances and found a direct inverse relationship between these two variables. A theoretical interpretation of these data led him to conclude that the "normal" intracellular environment is hypertonic and the cell expends considerable energy from respiration "pumping" water out to maintain its internal hypertonicity. Robinson has continued these studies and in two papers recently (167, 168) has presented data supporting his theory. Respiration in the slices was depressed with the mercurial mercaptomerin (Thiomerin), and in an attempt to study water changes independently from movement of ions the sodium chloride of the medium was variously replaced with choline chloride, sodium sulfate, or choline sulfate. The relationship between water content and respiration of slices held, despite these maneuvers to dissociate common ion movement. Even when the external medium was replaced with liquid paraffin so as to eliminate external osmotic pressure completely as a factor, anaerobic slices extruded "water" into the paraffin medium when oxygenation was begun. Deyrup (169) has come to similar conclusions from a slightly different approach; she found that renal slices were able to regulate their fluid and electrolyte content remarkably well even in the face of gross distortions of the composition and tonicity of the surrounding medium. Robinson has not directly measured ion fluxes in any of his studies and, although very ingenious and convincing, they would be more so if he had followed sodium and potassium in slice and medium. Deyrup has done this. Certainly without an exhaustive consideration of the subject one must conclude with Robinson that "the question of active transport of water is still open."

A number of papers have appeared which deal with measurement of various body spaces and their composition (170 to 178) and with various aspects of electrolyte excretion (179 to 189) which will not be discussed here. Rothstein (190) has continued his studies of the cation binding properties of the yeast cell surface and finds that the binding of UO_2^{++} is rapid and reversible and obeys a simple mass law equation. He postulates at least two binding sites as phosphoryl and carboxyl groups located at the cell periphery and isolated from endogenous cations by a permeability barrier. Rothstein's work continues to write an important chapter in the role of the surface "binding sites" as carriers of glucose and ions. Opie also has continued his studies of cell permeability and ion transport *in vitro* in three important papers this year (191, 192, 193). Aach, Rolf & White have presented a detailed and careful study of water losses and gains in fasting and nephrectomized rats (194) which will serve as excellent reference material for those studying chemical morphology following nephrectomy.

AUTOREGULATION

The Pappenheimer & Kinter theory (14, 195) has raised a great deal of renewed interest in autoregulation. Although not yet published in definite form, several studies have been presented this year seriously questioning the adequacy of this theory. Since these contrary data constitute one of the main "issues" in renal physiology at present, they should be mentioned here if this review is to reflect at all the present status of the field. The Pappenheimer-Kinter theory would predict that the degree of red cell "skimming" depends on arterial pressure, and since PAH extraction would also be related to the hematocrit of the blood perfusing tubular tissue, PAH extraction should vary with renal arterial blood pressure. Thompson, Kavalier & Lozano (196), however, found in dogs that PAH extraction remains constant over a wide range of renal arterial perfusion pressures (60 to 140 mm. Hg.). In two dogs extraction of PAH did fall in acute anemia; thus to this extent their findings agreed with the Pappenheimer & Kinter idea. Kessler, Heidenreich & Pitts (197), reasoning from the Pappenheimer-Kinter thesis, concluded that it should predict an increased splay to the glucose titration curve in the kidney during acute anemia because of changes in filtration rates of individual glomeruli in different areas of the kidney as the skimming phenomenon disappears with reduction in hematocrit. However, no significant difference in splay of the glucose titration curve was found in acutely anemic dogs, and thus the results were felt to be inconsistent with the theory. It is hoped that this interesting and imaginative theory will continue to evoke research that challenges it, for only in this way does our thinking progress, and certainly no theory is ever sacrosanct; but if it has made us seriously re-examine a field, it has served a real purpose. Certainly this is what Pappenheimer & Kinter have done for contemporary renal physiology.

COMPARATIVE PHYSIOLOGY

During their year in the Sahara (1953 to 54) the Schmidt-Nielsen made important and ingenious studies on the overall water economy of the camel.

They reported observations on body temperature of the camel and its relation to water economy (198). Besides being able to withstand a greater degree of total desiccation and to conserve water in kidneys more than most animals, the camel also relieves himself of the necessity of evaporating water to keep his body temperature down. In the hot months he allows his body temperature to rise; these wide diurnal swings in temperature iron out in the colder months or if the camel is given water *ad libitum* during the heat. The donkey also shows variations of temperature but not so wide as the camel, nor is he able to withstand extreme desiccation as can the camel. A striking finding (199) is that the camel, unlike nonruminants, shows a marked reduction in urea excretion and urea clearance when on a low-protein diet. It is known that ruminants are able to utilize urea nitrogen for protein synthesis by their rumen bacteria. The authors relate the enhanced urea conservation on a low-protein diet (perhaps a bit too teleologically) to the need of these bacteria for increased urea in the absence of exogenous protein. The authors invoke changes in urea permeability or an active tubular secretion of urea in explaining these changing tubular conservation phenomena with changing dietary protein. Macfarlane, Mullis & Howard (200) have studied water economy in tropical merino sheep in Australia. The merino approaches the camel in water-conservation ability; it allows fluctuations in temperature but not so great as the camel. The merino achieves relative temperature stability by panting and hence loses water more rapidly than the camel who lets his temperature go up higher. The merino, however, is able to withstand body desiccation to almost the same extent as the camel. The sheep is also like the camel in being able to adjust urea excretion to changing protein intake as the Schmidt-Nielsens have just recently shown (201).

Corcoran and associates (202), studying osmotic diuresis in the rat, showed that this animal has a very effective water-conserving mechanism. Standing in the order of their relative capacities to concentrate the urine from least to most are: man, dog, rat, and kangaroo rat. Similar studies have been done on the kangaroo rat by the Schmidt-Nielsens (203) but without mannitol loading. Jørgensen and associates (204) found in the toad, *Bufo bufo*, that water balance principle is liberated from the neurohypophysis during dehydration, but the cutaneous and renal responses to dehydration so far have been found to be independent of this neurohypophyseal activity. Lowrance, Nickel, Smythe & Bradley (205) wondered whether the "dive reflex" in the harbor seal (renal function shut down during diving) results from anoxemia alone or also from asphyxia. Therefore they studied renal functions in the seal during anoxic anoxia. They concluded that apnea (asphyxia) and anoxia have similar effects in inducing the "diving reflex" changes.

CONCLUSION

The field of renal physiology and water and electrolyte metabolism is really several fields at the present time and is too broad to be completely encompassed in one review. The author, therefore, has been somewhat ar-

bitrary in dealing with his assignment and has tended to place most emphasis on those subjects that interest him most. In surveying this vast area one is struck with the possibilities it offers for penetrating almost any area of physiology. One must understand cellular metabolism, enzyme chemistry, and thermodynamics to gain a clear picture of biological transport. The endocrine system enters as one considers antidiuretic hormone or aldosterone; the nervous system is explored both centrally and peripherally as we attempt to localize diencephalic osmoreceptors or thoracic volume receptor afferents. We can know the approaches of the cardiovascular physiologist and study renal blood flow and the phenomenon of its autoregulation; we must have available isotope techniques to study the forms of transported substances, the intermediary steps and the kinetics. Above all we have the opportunity in this field, as in all others, to display versatility and imagination in utilizing whatever methods and new theoretical approaches that are needed to advance our understanding of the problem confronting us. A review of the field at present leaves one with the overwhelming impression of the extent to which this is being done.

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DIGESTION¹

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Every reviewer in any field must develop a feeling of resignation to the task of covering his field completely. A large number of papers cannot be reviewed because of language difficulties. This is one of the great problems in all science. Numerous papers are in the area between his subject and adjacent fields, producing the problem of sifting. The number of papers falling into this group is very large in the case of digestion and the sifting must be dependent upon the reviewer's own interest and knowledge. As a matter of fact the Digestive System is an entity no more than in the anatomical location of the events. Physiologically it is far from a unit. The processes of secretion in salivary glands, stomach or pancreas are more closely related to events in the tubular cells of the kidneys or the formation of aqueous humor in the eye than to the motility of the different parts of the digestive canal. Scientists interested in motility find it more profitable to keep up with the progress in general muscle physiology than with the biochemistry of absorption processes in the intestine.

In order to bring together things which are related I have chosen a physiological organization instead of an anatomical one. This approach was also used in the review by Code in 1953 (1). I feel, however, my inadequacy in certain areas because of the heterogeneity of the subject. It would be better in the future to divide "digestion" into two or more topics; at least the reader would benefit from it.

The general state of gastroenterological research is in some respect one of stagnation and those papers indicating new avenues of approach should be fully appreciated by the scientists in the field.

On the subject of the physiology of gastric secretion James (2) has written a very detailed monograph. Even if his interpretations of the experimental facts in several respects differ from my own (3), it is a pleasure to recommend this book to everybody interested in this field. The physiological treatment in another review (4) does not seem to be in concord with established knowledge.

Lagerlöf (5) has given a review on the physiology of the pancreas.

SECRETION OF IONS AND RELATED PHENOMENA

Water transport.—Most of the authors reporting on volume secretion have not investigated water transport mechanisms. They are more concerned with the amount of juice secreted, where it comes from and how it is stimulated. Schneyer *et al.* (7), who earlier reported (6) that only 60 per cent of the total

¹ The survey of literature pertaining to this review was completed in July, 1957, but at that time the June or even the May numbers of some journals especially from foreign countries had not been received.

mixed saliva came from the big glands, reinvestigated the problem and found that almost everything comes from the large glands, viz. 69 per cent from the submaxillary glands, 26 from the parotid and five from the sublingual glands. A representative value for resting total saliva in man is 0.35 to 0.38 ml./min.

To determine the volume of gastric secretion in man Liebowitz *et al.* (8, 9) introduced $Zr^{95}-Nb^{95}$ as a diluting indicator. They found surprisingly high residual volumes in the stomach (3 to 83 ml.), but these values should be accepted with caution because they used a large water solution as a vehicle for the radioactive material, which may facilitate duodenal regurgitation. For the same reason their volume responses after histamine, almost double that usually reported, require more controls.

The 24 hr. volume of pancreatic juice varies between 2400 ml. down to 4 ml. (10).

Durbin, Frank & Solomon (11) investigated the water transport in the isolated gastric mucosa of the frog with the help of tritiated water. They observed an active net transport of water in the same direction as the HCl secretion. In the absence of an osmotic gradient this net flux equaled 11.3 $\mu\text{l.}/\text{cm.}^2/\text{hr.}$ A permeability coefficient of 174 $\mu\text{l.}/\text{cm.}^2/\text{hr.}$ was obtained. They do not state, however, in which direction the coefficient was measured, a point of importance if a net transport of water occurs. Fortunately in this particular case the small net transport will have a very limited influence. In experiments with mouse stomachs where the net transport was hydrostatically balanced to zero Öbrink obtained a permeability coefficient of 69 $\mu\text{l.}/\text{cm.}^2/\text{hr.}$ (12). Durbin *et al.* state that the osmotic flux mainly depended on a bulk flow, which is in opposition to Chinard (13) who stated that every flux dependent on activity gradients across a semipermeable membrane is a result of diffusion.

Assuming no interaction between the diffusing components within the membrane the activity gradients of water may result in a net flux and Öbrink (12) found this postulated net flux large enough to explain the volume secretion in a stimulated gastric mucosa.

If the Harvard group (11) have interpreted their results correctly the mucous membrane should contain pores with a radius of 2.5 Å and 60 Å (93 and 7 per cent of the pores respectively).

Öbrink (12) pointed out that work with labelled water gives results that probably differ from the values for ordinary water. Reitemeier, Code & Orvis (14), continuing their studies on the water absorption in man, showed that the initial slope of the relation between the percentage of absorption and time in the intestine gave 20 per cent/min. for HDO and 10 per cent./min. for Na^{22} . A very interesting method and interesting results were reported by Smyth & Taylor (15), who found the transport of water from the mucosal to the serosal side, *in vitro*, to be dependent on the glucose concentration and to have an optimal temperature of 40°C. The process is oxygen dependent and can be inhibited by different metabolic poisons.

Goth, Nadell & Edelman (16) found that 12 per cent of the total body water of the rabbit was in the digestive canal viz. four per cent in the stomach, two in the small intestine and six in the colon. These are only static figures for a highly dynamic system.

With the method described by Opie (17), Mallik & Ganguly (18) determined the osmotic pressure in the mucosal cells of the stomach of rat and guinea pig. The cells of the rat were in equilibrium with a 250 m.eq./l. solution of NaCl and those of the guinea pig with a 225 m.eq./l. The fact that they do not swell *in vivo* is then supposed to depend on an active transport of water. The authors discuss the failure of that pump mechanism as a possible cause of peptic ulcer. Riecker, Zack & Renschler (19), using the same technique obtained isogravity for the rat liver at 0.21 to 0.32 moles/l. NaCl (in water-deprived rats values as high as 0.60 moles/l). It may be disputed whether these values really represent intracellular osmotic pressures.

Wilson (20, 21) found that both water and NaCl move from mucosa to serosa in the intestine, but that this movement is inhibited under anaerobic conditions.

Perry (22) demonstrated that the terminal ileum in cat can absorb large amounts of water—more than the whole colon. That the water leaves the intestines via the portal system and not via the lymph was again clearly demonstrated by Benson Jr. *et al.* (23). The net absorption of water, sodium and chloride in the small intestine was analyzed also by McHardy & Parsons (24). In patients with nontropical sprue the absorption of the water and sodium was very much decreased (25), as was the unidirectional flux of deuterated water and radiosodium from the lumen in cases of inhibited motility [Higgins, Code & Orvis (26).]

Hydrogen ion transport.—Although hydrogen ion transport is involved in the pH changes in saliva, gastric, pancreatic, and intestinal juice it is most exaggerated in the stomach. No agreement has yet been reached as to where the acid in the stomach is formed. It is therefore of considerable interest that Wright, Florey & Sanders (27) in their experiments on different reptiles showed histologically that no parietal cells are present but only cells similar to the peptic cells. In spite of this the pH of the gastric juice was about 1.0. This approach should be a fruitful one. Also the work of Marks (28) should be used for continuous observations because it has shown a way of changing the number of the parietal cells. This was done by injection of histamine in wax in guinea pigs. Normally the number increases with the body weight, but here a decrease occurred between the second to fourth and the thirteenth to sixteenth week. A combination of this method with a quantitative determination of the *maximal* secretion capacity of hydrochloric acid would give valuable information. Hill (29) reported a relationship between the development of parietal cells and appearance of HCl. Ebers *et al.* (30) found high acidity in newborns a few hours after birth.

Davenport (31) found the efficiency of the mouse stomach in producing HCl in aerobic condition to be 10 per cent with pyruvate and 11 per cent

with acetoacetate. In his work with Chavré (32) on the rôle of succinoxidase, which is in high concentration in the parietal cells, he concludes that the failure of malonate to inhibit gastric secretion might be due to its inability to reach a sufficiently high concentration in the gastric mucosa. Another succinoxidase inhibitor, antimycin A, readily inhibited the gastric secretion in frog and mouse stomachs *in vitro* but only in frogs when used *in vivo*. The formation of two moles of lactic acid contributes the same amount of energy for hydrochloric acid production as the consumption of one mole of oxygen, which suggests a rather low efficiency for the secretory process (32).

Vitale *et al.* (33) also showed that the parietal cells have a high concentration of succinic oxidase and that this enzyme system is stimulated by histamine, while the malic dehydrogenase is not. This is particularly interesting as the rôle of histamine is very obscure.

For present concepts on the carbonic anhydrase and gastric secretion see (34, 35, 36).

Hollander (37) tested some enzyme inhibitors on Heidenhain dogs. Iodoacetamide and N-ethyl maleimide completely inhibited histamine induced gastric secretion. Contrary to the case of *in vitro* experiments (38) this inhibition is completely reversible, which is not surprising. Douthwaite & Booth (39) tried the inhibiting effect of mercalyl on the gastric secretion in nine patients.

Rehm has continued his important approach on the influence of electric currents on the secretion of HCl. Earlier (40) he showed that a current from serosa to mucosa increased the H^+ -output, whereas if applied in the opposite direction it decreased it. Now (41) he demonstrates an opposite tendency for the chloride ions, which he explains as interference with the intramucosal currents generated from an active chloride transport. In this theory the chloride ions are actively transported across the parietal cells whereas the hydrogen ions are secreted from the surface epithelial cells. However, a simple electrolysis must be excluded and the direction of the "chloride battery" might be a matter of discussion. It may be interesting to note that Logothetopoulos & Myant (42) in an elegant piece of work on hamster, mice, rats, and guinea pigs with an autoradiographic technique showed that iodide and thiocyanate accumulate in the region of the surface epithelial cells. If these results could be extended to other halogens as well it would be in better agreement with Rehm's first hypothesis that the chloride ions were secreted there (43). Rehm and coworkers also continued the measurements of potentials and impedances in the resting and secreting gastric mucosa (44, 45).

In Conway's laboratory the study on ion transport in yeast has continued. In a paper with Duggan (46) it was shown that the cation-carrier in the wall of the cells carried 0.12 m.eq. of K^+ per kg yeast. The carrier probably contains iron.

The method to detect free acid in the stomach by determining chinin in

the urine, split free from a cation exchanger, was used by Kromrey (47). A similar approach with methylene blue in bags closed with catgut was unsuccessful (48), but the method should be further developed and more reliable controls developed. Demole (49) got a temporary decrease in free acidity with amino acids in the stomach, and interestingly Hunt (50) found a decreased output of HCl if hydrochloric acid was added to the test meal.

Wilson & Kazyak (51) showed a bicarbonate accumulation in the jejunum and ileum of hamsters. They suggest that there exists an exchange transport in the mucosa of Na^+ towards the lumen for H^+ from the cellular water in the other direction. On the serosal side this causes an increase in CO_2 -tension and a decrease in bicarbonate. CO_2 diffuses through the mucosa and combines with the OH^- left from the water to form bicarbonate [c.f. also Parsons (52)].

Sodium and potassium ion transport.—Coats & Wright (53) found a basal secretion of 0.21 ml./min. from the parotid gland in sheep, but could increase this to 5.24 ml./min. by electrical stimulation of the nerve. A steady state was reached at a level of 2.64 ml./min. They found an increasing concentration of sodium with increasing secretion rates whereas potassium decreased. Chloride was constant but bicarbonate varied in the same way as sodium. The chloride and potassium figures are a little surprising as it is usual to find a relatively constant potassium concentration, and the chloride usually varies inversely with the bicarbonate. The normally-obtained relationship between cation concentration and secretion rate was reported by Denton (54), who found that the Na^+/K^+ quotient varied directly with the secretion rate between 0.1 to 3.0 ml./min. At higher rates the quotient became more constant. In sodium-depleted sheep the quotient decreased. After a close intra-arterial infusion of acetylcholine, a marked increase in volume occurred. This caused no increase in the Na^+/K^+ quotient in normal animals but a significant one in the Na-depleted. In the reviewer's opinion, this may depend on the hyperbolic relationship between the concentration of sodium and the secretion rate which was clearly shown by Thorn, Swartz & Thaysen (55) in man. They found the maximal Na^+ concentrations to decrease in patients with restricted Na^+ -supply. They also found an inverse relation between K^+ and secretion rate. They reported 18.0 mN and 26.2 mN as maximal values for normal and sodium-depleted patients respectively.

These high values for K^+ in the saliva as well as the variations according to the secretion rate are problems that have been treated in several papers on the stomach. Some authors (56, 57) found constant K^+ values uninfluenced by the secretion rate—whereas Hollander (58) and others (c.f. 3) found a definite rise in the K^+ concentration at the start of a secretion period. I think that the different results have been explained by the brilliant works of Burgen (59). He found an initially increased outflux of K^+ from the parotid and submaxillary glands in dogs. It was derived from the glands themselves and not from the blood. Surprisingly, also the blood gained potassium so

that the concentration of K^+ in the venous blood was higher than in the arterial. The gland lost 20 m.eq./gm./min. to the saliva and 4 m.eq./gm./min. to the blood. After a short period the potassium flux between gland and blood changed, so that the gland started to take up K^+ from the blood. After cessation of secretion the gland continued to take up K^+ for about 80 min. With a suspicion of an active transport mechanism for K^+ Burgen varied the temperature and found $Q_{10} = 1.6 - 2.0$.

Probably directly related to the above mentioned transitory potassium losses are the findings reported by McMillan & Vane (60), that histamine (and acetylcholine and $BaCl_2$) temporarily increased the plasma potassium in cats from 3.2 to 4.6 m.eq./l. A concomitant decrease in blood corpuscular potassium was observed.

The effect of the choline esterase inhibitor (isopropylmethyl-phosphonofluoridate; Sarin), that was reported by Wills & Somers (61) should be compared to the findings of Burgen. The inhibitor as well as acetylcholine decreased the potassium uptake by the gland. Sodium and potassium concentrations in the pancreatic juice were roughly constant at 140 and 9 m.eq./l. respectively. Diamox lowered the secretion rate but did not disturb the concentration-volume relationship [Dreiling & Janowitz (62)]. These results were confirmed in dogs by Bro-Rasmussen, Killman & Thyssen (63). Their results were compared with similar results from the lacrimal, and parotid secretion as well as with that of sweat.

Reitemeier, Code & Orvis (64) found a very poor permeability for sodium through the gastric mucosa from instilled solutions, but whether this invalidates the earlier findings that sodium is freely permeable through the isolated mucosa (65) cannot be judged.

Remembering that Bowie *et al.* (66) could inhibit the gastric secretion by topically applied NaF, Bond & Hunt (67) instilled five M NaF into the stomachs of anesthetized cats stimulated with histamine and tried to learn whether this inhibition of acidity depended on increased neutralization or diffusion. They added radiosodium and found, like Code & coworkers (64), that the mucosa was relatively impermeable for the sodium ions. After treatment with NaF the influx of Na^+ increased and the hydrogen ions disappeared ten times faster than before. Although no definite explanation of the results can be presented the observations are of fundamental importance and further work along this line will be profitable.

The resorption of Na^+ in jejunum was greater with HCO_3^- , Cl^- , CH_3COO^- or NO_3^- than with SO_4^{--} , but in ileum there was a slow resorption with bicarbonate [Budolfson (68)]. See (51) where it was shown that we have an active transport of bicarbonate towards the mucosa.

Sodium and potassium concentrations in bile of the bladder post mortem were 169 and 27 m.eq./l. as average values respectively, but these values may be different from living conditions (69).

Anion transport.—Present interest in the halogens of the digestive tract

is great. To analyze the chloride secretion mechanisms is very difficult and only a few attempts have been made (70). Another approach has been to study the behavior of other halogens such as Br and I.

Fletcher & Rowlands (71) found a five- to ten-fold accumulation of radioiodide in slices of salivary glands from mice. Only inorganic iodide was accumulated. This accumulation occurred at medium-low concentrations (less than 40 $\mu\text{g./ml.}$). Above that concentration the accumulation quotient was around one and never lower than 0.8. C.f. (72). Addition of Br^- had no effect on the accumulation but ClO_4^- , SCN^- and NO_3^- inhibited it. Carrier iodide had the same effect. The effect of these ions is probably one of competitive inhibition. As 2:4-dinitrophenol also inhibited the iodide transport, this is probably dependent on energy rich phosphate. The rôle of the endogenous thiocyanate was investigated but its rôle remains obscure.

Logothetopoulos & Myant (73) by using I^{131} and S^{35} -labeled thiocyanate found an accumulation in the submaxillary glands of hamster and mouse but not in the sublingual or parotid glands. In the rat there was no accumulation at all. Autoradiographic examinations confirmed the results. Gabrielsen & Kretchmar (74) studied the accumulation of iodide in the saliva of man. Inorganic iodide accumulation was independent of the thyroid status. When using quantitative measures one would like to be certain that the secretion rate did not vary since this may change the accumulation ratios. This is especially true in papers (75, 76), where average values of saliva chloride have been given for children. These figures are no more meaningful than other average values of concentrations that vary with the volume secretion rates. This also applies to a paper on concentration in gastric juice (77).

Logothetopoulos & Myant (42) found a very high accumulation of radioiodide and labelled thiocyanate in the stomach in hamsters, mice, rats and guinea pigs. Autoradiographic analysis showed that in the fundic part only that part of the surface down to the bottom of the foveolae was blackened. Thus iodide and thiocyanate were accumulated in the surface epithelial cells and no concentration was observed in the region of peptic or parietal cells. One is anxiously waiting for similar experiments with bromide and possibly chloride to judge where the possibly existing active anion transport through the gastric mucosa takes place.

That the work just mentioned (42) is not immediately applicable on chloride is made apparent by the work of Howell & Middlesworth (78). They studied the excretion of iodide and chloride in the gastric juice and expressed it in clearance values analogous to the kidney. Even if this is not entirely meaningful until one has analyzed "Tm" values that possibly exist for both the secretion and backdiffusion, the experiments are nevertheless extremely interesting. By using substances such as NaSCN and NaClO_4 that block the uptake of iodide in the thyroid the clearances for iodide were depressed from values 10 to 15 times that of chloride to values equal to chloride. The authors suggest two different transport systems for iodide: a

specific one and one similar to that for chloride. This may not be necessary if there is a competition about a common carrier mechanism (71, 79, 80, 81).

Budolfsen (68) showed in rat, that chloride absorption decreased in combination with cations in the following order: $\text{NH}_4\text{—Na—K—Ca—Mg}$ (colon) or $\text{Na—NH}_4\text{—Ca—K—Mg}$ (ileum).

Sweet, Nadell & Edelman (82) studied the total exchangeable chloride in the digestive tract, using Br^{82} . They considered Br^- and Cl^- to behave in the same way. As this is not the case (80) the figures 11.7 per cent for the stomach, 2.5 for the small intestine and 1.7 for the caecum and proximal part of the colon may have to be corrected.

Dreiling & Janowitz (62) found in man that the bicarbonate concentration in the pancreatic juice increased with the volume secretion rate while chloride varied inversely. The sum of bicarbonate and chloride was constant, about 144 mN [c.f. (63, 79)].

Osmotic pressure.—Thull & Rehm (83) found that increase in plasma osmolarity decreases the secretion rate and temporarily increases the osmolarity of the gastric juice. The H^+ ion concentration decreases and the Na^+ ion increases. K^+ ion does not change. Lowering the plasma osmolarity increases the secretion rate and causes a decrease in gastric juice osmolarity but changes the Na^+ and K^+ very little. The authors used a mixture of the secretion and the volume in the "pooling space" in order to get large volumes with rather low acidity values. This makes the interpretation of data extremely difficult and the reviewer cannot feel convinced that the interpretations are correct even though the approach is interesting. The only safe way would be to use steady state conditions.

Transport of miscellaneous substances.—McHardy & Parsons (84) studied the absorption of inorganic phosphate in the intestine and Gershoff & Hegsted (85) that of calcium. Jacobi *et al.* (86) found the interesting fact that iron ($\text{Fe}^{59}\text{Cl}_3$) is complexly carried through the intestinal mucosa by cysteine or ascorbic acid. Q_{10} of the process is more than 3. Lactose, L-lysine and L-arginine promoted the absorption of Ca and Sr (87).

Shore, Brody & Hogben (88) found a partition between gastric juice and plasma in dogs for several acidic and basic drugs that could be explained by a lipid structure of the membrane through which the substances could pass only in an unionized state. Thus the partition is dependent on the pH.

As urea penetrates the intestinal wall, intestinal loops were used for lavage in cases of uremia (89).

Hirvonen & Hartiala (90) tried 11 different dyes that are known to be eliminated in the stomach or the pancreas but none was eliminated in the duodenum.

The incorporation of Na_2SO_4 (S^{35}) in the duodenal secretions in rabbits was studied by Kent *et al.* (91) and Jennings & Florey (92). It was found in mucus producing cells, mucus substances and in unknown dialyzable groups.

The quantity and composition of the intestinal gas is influenced by many

as yet uncontrollable factors according to Askevold (93), who also determined the relative diffusion rates for the different gases.

SECRETION OF ENZYMES AND OTHER ORGANIC COMPONENTS

Salivary amylase.—Of all amylase in the saliva 2.4 per cent comes from the sublingual glands, 44.6 from the submaxillary and 53.5 from the parotid glands (94). The synthesis of amylase is increased by stimulation to salivary secretion [Schneyer & Schneyer (95, 96)].

Organic components in the saliva.—Leeson (97) found an alkaline phosphatase at the base of the acini. For the first time a low mobility electrophoretic fraction containing no glucoproteins was found in the parotid saliva [Drevon & Donikian (98)].

Hokin & Sherwin (99) found, that as in the pancreas stimulation with many different agents increased the incorporation of P^{32} in the phospholipides of the submaxillary gland. The fact that the turn over of P^{32} is in the water soluble part of the phospholipides but not in the fat soluble one may mean that the phosphoryl group is part of a carrier mechanism in the gland. Shein (100) strongly opposes the reports by Stern & Igliseas (101) that there is a uricolytic activity in the human parotid glands.

An important contribution was given by Burgen (102) who studied the kinetics of ten nonelectrolytes in the parotid saliva. The curves relating the concentration and secretion rate have different shapes. Some substances show an exponential decrease of the concentration for increasing secretion rates, whereas others show a U-form. From mathematical analyses he concludes that the glands contain a porous membrane, the permeability of which changes when the cells are activated. The diffusion barrier is the surface of the cells facing the lumen. The permeability coefficient is shown to change for those substances having a U-formed concentration-secretion rate relationship. This is explained by a porous theory with restricted diffusion.

Hilton & Lewis demonstrated that stimulation of the chorda tympani nerve induces a production of bradykinin and vasodilation and that this happens also after acetylcholine, epinephrine, and norepinephrine (103). In all cases bradykinin is formed. Therefore the vasodilation after chorda stimulation does not prove that the nerve contains vasodilating fibers.

Hoerman *et al.* (104) showed that lysozyme is formed by the submaxillary and the sublingual glands.

Colamin, that earlier was obtained only in patients with disturbed metabolism in liver disorders (105) was shown by Georgi & Honegger (106) to be present in normal mixed saliva together with ammonia, methylamine, and di-methylamine.

English (107) showed that radiation of salivary glands by 2000 r in otherwise protected rats showed an increase in glucose-6-phosphate-dehydrogenase but not in isocitric-dehydrogenase as was the case after whole body radiation (108).

Pepsin.—Moussa (109) considers the Golgi apparatus to be the center of enzyme formation in the peptic cells.

The effect of histamine on the pepsin secretion is still a matter of dispute. Hirschowitz *et al.* (110) think they have solved the problem in favor of histamine being a pepsin-stimulator, but in the reviewer's opinion the question is still open.

Perry *et al.* (111) very clearly demonstrated that in the case of ulcers the proteolytic activity in the gastric juice is increased rather than that the resistance to digestion is decreased. Klotz (112) used radioactive albumin in pepsin determination. On a resin column pepsin from man and cow appeared in the same peak (113). Rosen, Townsend & Seifter (114) succeeded in protecting Shay-rats from ulcer with alumina gel and sodium-polyanhydromannuronic acid, which like other sulphated polysaccharides inhibits the activity of pepsin.

Pepsinogen-uropepsin.—Adrenalectomy in rats reduced the pepsinogen content in the stomach. It was restored to normal values with cortical steroids. ACTH² increased it above normal. Varró *et al.* (115) report similar results in their studies on plasma pepsinogen. Hypophysectomy plus adrenalectomy was even more potent in reducing the pepsinogen [Hirschowitz *et al.* (116)].

The excretion of uropepsin that takes place by glomerular filtration (117) is a poor measure of the secretory activity in the stomach (118). The uropepsin excretion is possibly related to the amount of normal mucosal tissue (118). Only in pernicious anemia and total gastrectomy was the uropepsin absent (119). In some patients with postcibal symptoms an increased excretion was found (120) but as a conclusion it can be said that our knowledge about uropepsin is still scant. For literature see (121).

Organic components in the gastric juice.—Separation of constituents of gastric juice on Amberlite IRC-50 columns yielded five different proteins and two polysaccharides (113, 122). The method was used also in some clinical work by the same group [Wolf, *et al.* (123)].

Using electrophoretic analyses of gastric juice Norpoth *et al.* (124) found that the results depended on the method of concentrating. Glass and his coworkers (125) succeeded in developing a reliable method for paper electrophoresis. The peaks were named P (pepsin), M₁, M₂, M₃, M₄ (mucoproteins), X, Y and Z (unidentified). People with pernicious anemia and mucosal atrophy lack the peaks P, M₁, Y and Z and have only a small X. In one case with anacidity but retained intrinsic factor, M₁ was present.

Hartila showed that the gastric and intestinal mucosa has a very high glucuronide synthesizing activity [c.f. (14)] but Brummer *et al.* (126), who found a decrease in the indican excretion after HCl administration in patients with achlorhydria, showed that this was not due to changes in excretion of glucuronic acid or glucuronides. The excretion of phenols was not influenced (127).

Organic components in the pancreatic juice.—Babkin and his co-workers

long ago formulated a theory according to which all the three principal enzymes were secreted in parallel and that the concentration of protease was proportional to the protein content of the secretion. Neither of these theories could be confirmed in a very extensive and detailed work by Guth, and his co-workers (128) nor was there any adaptive change in the relative concentrations of the enzymes to the kind of food given. Their paper should be consulted for determinations of enzyme activities and kinetics.

Rothschild & Junqueira (129) using paper electrophoresis on rat pancreatic juice found seven fractions. One of them contained chymotrypsin that was already partly activated. By countercurrent electrophoresis the demonstrated heterogeneity of alpha-chymotrypsin was shown to depend on the presence of beta- and gamma-chymotrypsin [Egan *et al.* (130)].

During stimulation there is an increased protein synthesis (131) and several amino acids like phenylalanine, alanine, histidine, and methionine are incorporated in the pancreatic proteins (132). Peptides have been found to stimulate the enzyme synthesis (133). After pancreozymin but not after secretin there is an increased turnover of phosphate in the phospholipids in the pancreas of pigeons and this turnover is probably related to the secretion of enzymes [Hokin & Hokin (134)]. Yamashina (135) demonstrated that enterokinase activated trypsinogen to trypsin by splitting off a valyl-peptide at the end of the molecule.

Direct function tests on the pancreas have always been difficult to develop, but several attempts have been made to study the function indirectly. By use of I^{131} -labeled fat it was shown that after pancreatectomy or in pancreatitis the resorption of radioiodine was normal for oleic acid but decreased for triolein (136). The same group (137), used this technique in clinical diagnosis, but the determination of radioiodide may be invalidated by trapping by different tissues. Iodated albumin was used by Faber *et al.* (138) to examine the proteolytic activity in the digestive tube and they found that gastrectomy did not influence the absorption of iodide.

Lipase *per os* increases the resorption of lipoids in healthy subjects (139). Ivy *et al.* concluded that the intestinal lipase mainly hydrolyzes esters of unsaturated fatty acids (140) while the pancreatic lipase attacks both saturated and unsaturated fats (140, 141, 142).

STIMULATION

Salivary glands.—Emmelin & Strömblad (143) succeeded in making the submaxillary gland in cat supersensitive to adrenaline with intraductal application of piperidino-aethyl-diphenyl-azetamid which is a kind post-ganglionic pharmacological denervation without interfering with the acetylcholine formation.

To obtain data on the relative importance of the parasympathetic nervous system in the salivary glands Snell & Garrett (144) determined the choline esterase in the submaxillary and sublingual glands in the rat. It was present in the duct cells and around but not in the acinar cells.

Salivary glands and the endocrine system.—Kahlson & Renvall (145) showed that hypophysectomy or bilateral adrenalectomy causes an atrophy of the submaxillary glands. The acinar cells of the parotid gland became smaller and the amylase secretion reduced [Baker *et al.* (146)]. Shafer *et al.* (147) showed that a combination of thyroxine and testosterone is effective in preventing this atrophy. The protease activity was also restored (147) as well as the concentration of ribonucleic acid [Bixler *et al.* (148)]. The disappearance of the thyroid function resulted in a progressive disappearance of RNA in the submaxillary and sublingual glands in rats (148) [c.f. also (149)]. A mutual interaction seems to be present as Wase & Feng (150) found that removal of the salivary glands depressed the thyroid activity.

Stomach.—Miller & Haszyc (151) demonstrated an increase in number of blood-filled capillaries in the gastric mucosa of man after histamine or ACTH. Nordgren & Öbrink (152) stated, however, that histamine does not exert its stimulating effect by vasodilation, as a vasodilator (prisco) did not stimulate, but only enhanced an already existing secretion.

Woodward *et al.* (153) showed that alcohol acted as a strong gastrin liberator in antrum. Direct contact with the fundic cells did not provoke secretion.

Dragstedt and his group (154) showed that the amount secreted is proportional to the amount of mucosa present.

Gillespie (155) found that lowered body temperature decreased the secretion rate after histamine and the acidity even more. He did not, however, investigate whether the normal relationship between secretion rate and acidity was disturbed, but one gets the feeling that this was the case. Berenblum & Fogel-Kaufman (156, 157) developed a method for using rats and mice in secretion experiments and tested histamine, atropine, acetylcholine, and pilocarpine. Wright & Trethewie (158) found a high concentration of histamine in reptilian stomachs.

The question whether histamine is a normal mediator for the gastric secretion was touched upon by Vitale *et al.* (33) and indirectly by Lin *et al.* (159, 160), who used a histaminase inhibitor, aminoguanidine, in dogs. Under certain conditions the secretion of HCl increased. Fraile-Ovejero & Luch-Trull (161) observed similar effects in cats.

In 1949 Linde (162) succeeded in inhibiting gastric secretion by intravenous injections of antihistamine. In carefully performed work Kay & Forrest (163, 164) could inhibit the acid output in Heidenhain dogs by irrigation with the antihistamine promethazine. This happened both after histamine and carbaminoyl-beta-methyl choline stimulation. It is interesting to note the disparity in volume and acid output, which they obtained.

Kirsner & Ford (165, 166) found a strong stimulating effect by intravenously administered reserpin, which was unaffected by vagus or adrenocortical stimulation. Atropine, banthine, or epinephrine had no effect on this hypersecretion (167). For an extensive review on histamine and gastric secretion the reader is referred to Code (168).

Stomach and vagus stimulation.—Agostoni *et al.* (169) have performed a

functional and morphological study on the vagus nerves in cats. Among other facts they stated that the anterior and posterior abdominal vagus passing through the diaphragm consists of about 31,000 fibers, less than ten per cent of which are efferent. In works on dogs Griffith & Harkins (170) demonstrated that if a vagotomy is made upon the branches passing into the stomach at the lesser curvature the benefit of a vagotomy is obtained without the unwanted side effects.

That the stimulating effect of insulin is due to hypoglycemia was demonstrated in pigeons by Koskowski & Mahfouz (171). Henning *et al.* (172), found a stimulating effect on secretion and motility of some of the new peroral drugs used in the therapy of diabetes mellitus.

Stomach and endocrine influences.—Kyle & Welbourn (173) found that hypophysectomy and adrenalectomy inhibits the secretion in pylorus ligated rats and according to Johnson Jr. *et al.* (174) sectioning of the pituitary stalk in dogs decreases the volume secretion. They found a disparity between volume and acidity but did not give data enough to analyze the results. They could restore the secretion to normal conditions with pitressin, which also is rather unexpected as this extract was known to inhibit the gastric secretion. Kyle & Welbourn (173) and Hartmann (175) restored the secretion after adrenalectomy with cortisone in low doses.

Norepinephrine (0.3–1.2 $\mu\text{g.}/\text{kg.}/\text{min.}$ always inhibited the secretion rate after histamine. This inhibition was probably not due to a vasoconstriction [Harries (176)].

Ivy & Lane (177) summarized some of the drug actions in the rat. Schurman & Kamen (178) used mice instead of rats for producing ulcers with the Shay-method.

Stomach and central nervous influences.—In an infant with a gastric fistula Engel *et al.* (179) found that outgoing affective mental states were associated with increases in secretion while inactivity depressed the secretion of HCl. The inhibiting effect of chlorpromazine seems to be of central origin according to Sun & Shay (180).

Brobeck (181) reports that a destruction of the hypothalamus may lead to hyperphagia. If the destruction is more lateral the animals no longer eat. In animals with the central part injured amphetamin changed the electrical activity from that region.

That the appetite and satiety is not a matter of an empty or full stomach was shown by Fleming & Leatherman (182) on parabiotic rats.

A trial to establish dose-response curves between the influences on various amounts of food and gastric secretion (183) was not too fruitful.

Lepkovsky *et al.* (184) analyzed the influence of the content in the stomach and intestine on food intake. Rats which were given food without water ate only two-thirds of what they otherwise consumed.

Several studies on the effect of anticholinergic substances on gastric secretion have appeared. Janowitz & Hollander (185) found a 96 per cent inhibition in the third hour after administration of 0.4 mg./kg. atropine. Once

more they observed a disparity between secretion rate and acidity. Hexamethonium and atropine have a synergistic inhibiting effect [Kay & Smith (186)]. Bremer (187) using pentamethonium solutions concluded that the intramural nervous system plays an important role in the production of gastric juice. A marked inhibition was also obtained by the new anticholinergic drug N-ethyl-3-piperidyl-benzilate methobromide (Piptal) (188) and also with oxyphenonium bromide (Antrenyl) on augmented secretion after prednisone (189).

Humoral stimulation of gastric juice.—Uvnäs and his group (190) continued the investigation of the part played by antrum on the gastric secretion. They showed that exclusion of the antral mucosa from the acid increases the gastric secretion. They suggested different possibilities to explain this: increased gastrin liberation, disappearance of an inhibiting mechanism or a disturbed relationship in the cooperation between gastrin and vagus impulses. Waddell (191) discussed the nervous relationship between the antral and fundic mucosa when he found that vagotomy plus antrum exclusion and posterior gastroenterostomy inhibited the gastric secretion. In his case, however, the antral mucosa probably still had a rather low pH, and Kay (192) concluded that if pH in the antrum is below 5 (e.g., 2.5) the secretion is blocked.

The secretion increased after gastroileostomy (193) probably for the same reason as in Uvnäs' case.

For a decision of the three abovementioned possibilities the works of Jordan & Sand (194) and Harrison *et al.* (195) should be consulted. They demonstrated that there really exists an inhibiting mechanism in the antral mucosa. The nature of that mechanism remains to be shown, but it is interesting to note that in the work of Jordan & Sand (194) there was a latency period of one to three hr. for the inhibition. This was also the case with pure enterogastrone preparations (196). Further evidence that there is an inhibiting mechanism in the antrum comes from Brackney, Thal & Wangenstein (197).

Pancreas.—A continuous secretion from the pancreas was observed in the rat by Hirsch *et al.* (198), who explained it as an overloading of granulae in the cells giving rise to an overpressure that induced a discharge of the content. This explanation needs further experimental evidence before being acceptable. The secretion in the pancreas always causes a higher pressure in the pancreatic duct compared to the biliary duct (199).

Baker, Reid & Thoms (200) showed that hypophysectomy in rats causes a decrease in the weight of the pancreas and a depression of the proteinase activity (cf. the salivary glands).

The effect and importance of secretin, pancreozymin, and cholecystokinin was reviewed by Jorpes & Mutt (201). A combination of secretin and pancreozymin for obtaining amylase and lipase was used in man in chronic pancreatitis (202).

Lin & Grossman (203) got a straight line dose response relationship between the amylase output and the logarithm of pancreozymin injection.

Glucagon (204), atropine, probanthine, dactil, epinephrine, glucose, dextran, and other pharmaceuticals (205) inhibited the secretin induced pancreatic secretion.

Tankel, Hollander & Lester (206) showed that histamine possesses a stimulating effect on the pancreas, that can be inhibited by atropine. As histamine might induce pancreatic secretion indirectly by gastric acid running down into the duodenum and there liberating secretin, the experiments were performed with a gastrostomy to drain off the gastric acid. In order to be even more certain Tankel *et al.* (207) repeated the experiments after total gastrectomies and still got the same stimulation.

Pfeffer & Hinton (208) using secretin found that epinephrine depressed both the volume and bicarbonate secretion, and so did probanthine, whereas norepinephrine, ACTH and hydrocortisone had very little effect.

Tankel & Hollander (209) doubt that an increased blood flow *per se* should be the cause of an increased pancreatic secretion [c.f. the stomach (152)].

Injection of Zn^{65} gives a pancreatic radioactivity after 15 minutes higher than that in the liver. Very little appears in the duodenum (210).

Crider *et al.* (211) confirmed that hyperglycemia causes an increment of the enzyme output from the pancreas.

Liver.—Histamine causes an increase in both the liver weight and the intestinal weight whereas norepinephrine has the opposite effect, which McLean *et al.* ascribe to vascular effects (212).

Eriksson (213) found that thyroid function had an effect on bile cholesterol opposite to that on plasma cholesterol. The formation of cholic and chenodesoxycholic acid in hypothyroidism is decreased. In hyperthyroidism there is a decrease of taurocholic acid but an increase in taurochenodesoxycholic acid.

In bile duct cannulated rats there is a 12 hr. excretion of preformed bile acids. Then a ten- to twenty-fold increase in the synthesis was observed (214).

ABSORPTION

Absorption of glucose.—Wilson (21) described a modified technique in studying intestinal absorption. He used inverted intestinal loops from hamster and circulated a bicarbonate solution on the serosal side. Glucose was concentrated on the serosal side 75 times, lactic acid 4, and methionin 10 times. Water was transported towards the serosa against a pressure of 4 cm. H_2O . At higher pressure differences water went in the opposite direction and a flow of 20 $\mu l./mg./dry\ weight/hr.$ reduced the glucose transport to zero.

Reynell & Spray (215) found that the Cori technique (216) in obtaining a kind of "Tm" for the intestinal absorption is not accurate since a loading with glucose solution delays the gastric emptying and part of the glucose is trapped in the stomach.

Reynell & Spray (217) found that 90 to 95 per cent of the glucose absorption takes place in the first half of the small intestine in rats. Most of the iodide was absorbed in the distal part.

That the glucose absorption is dependent on oxygen was again demonstrated in a semiquantitative way by Cordier & Worbe (218), but the transport is probably both a specific one and a bulk transport. At least it is partly proportional to the rate of water absorption [Fullerton & Parsons (219)]. Xylose is transported only in the latter way.

Phlorizin inhibition of the absorption was not due to an inhibition of the alkaline phosphatase according to Jervis *et al.* (220). Adrenalectomy depressed the flux of actively transported carbohydrates [Vidal-Sivilla (221)], but such detergents as sulphonated lauryl alcohol in certain concentrations increased the absorption more than 100 per cent [Mosinger & Kozlik (222)].

Regarding the fate of the absorbed glucose Atkinson *et al.* (223) found 70 to 80 per cent of the absorbed glucose in the mesenteric veins. Most of the sugar is absorbed without degradation and the part that is degraded is used to give energy for the transportation [Hawkins & Wills (224)]. Also Taylor & Langdon (225) found it transported to the liver as hexose. Wilson (226) whose investigation agrees with the above mentioned suggests, however, that part of the glucose is converted to lactic acid. The production of lactic acid and transport of most of it to the serosal side offers a possible mechanism for the normal transportation of glucose.

In normal mice there is no further increase in the absorption of glucose if the loading surpasses 100 mg., but in obese animals the absorption is increased [Mayer & Yannoni (227)].

Fisher (228) showed that diarrhea normally appearing on prolonged feeding with lactose disappears when beta-D-galactosidase increases.

Absorption of amino acids.—That there is an active transport of amino acids through the intestinal mucosa was demonstrated by Wiseman (229) and Agar *et al.* (230). The latter showed that L-histidine is taken up actively but then diffuses out of the cells passively without any energy supply. D- and L-amino acids seem to be transported differently. The absorption of glutamic and aspartic acid partly involves a transamination [Neame & Wiseman (231)]. Free L-tryptophan is absorbed more rapidly than acetyl-D-tryptophan (232).

Absorption of fat.—Knoebel & Nasset (233) investigated the location of fat digestion and the composition of the tract after feeding. The absorption of acetic acid and glycerin from the stomach was analyzed by Hertin *et al.* (234).

As in the case of glucose absorption in studying fat absorption it is necessary to analyze the fat remaining in the stomach [Tidwell & Johnstone (235)]. Thomasson (236) suggested a method to describe how much fat disappears from the digestive tract, but the method suffers from the fact that the absorption is not uniform throughout as can be seen from other experiments (237) on absorption of vitamin A.

According to Morris (238) 30 per cent of the lymph in the thoracic duct

originates from the liver and the rest from the intestine. He also studied the origin of the proteins and lipides in the lymph. In rats Simmonds (239) showed that the formation of lymph and its propulsion in the thoracic duct during fat absorption was not influenced by the intestinal motility.

McKenna *et al.* (240) worked out a clinical method to determine the absorption of I^{131} -labelled glycerol trioleates in blood, feces, and urine. Malm *et al.* (241) showed a higher activity of radioiodide in the blood from feeding fat than from feeding fatty acids, which fits with the observation by Reiser & Dieckert (242) that a small part of tripalmitin is absorbed in an unhydrolyzed form. That glycerides can be absorbed through the mucosal cells without a complete hydrolysis seems to be definitely proven by Blomstrand *et al.* (243). Bergström & Blomstrand (244) studied the absorption of chimyl alcohol and found that three to five per cent was absorbed in 24 hr. The transportation of cholesterol from intestine to lymph takes place in an esterified form [Vahouny *et al.* (245)].

Adrenalectomy disturbs the fat resorption and cannot be restored by treatment with salt and DOC or cortisone (246).

MOTILITY

Swallowing.—Andrew (247) worked on the nervous control of the cervical oesophagus of the rat. He succeeded in differentiating between two types of efferents, the first type innervating the cricothyroid muscle which is relaxed for a short period during the start of swallowing, and the second type responsible for the propulsive wave. Also the afferents from different parts of the oesophagus were investigated. Atkinson *et al.* (248) likewise found that the cricopharyngeal sphincter, that normally is responsible for a zone of increased pressure, is relaxed during the act of swallowing. Their investigation that involves pressure measurements in three different levels and rapid series of x-ray radiograms was applied to clinical practice (249).

Muren (250) described an oesophageal fistula that enables the animals to eat normally without any great loss of food.

Cardia.—Allison (251) reviewed our present knowledge on the subject, and so did Lyons *et al.* (252).

Braasch & Ellis Jr. (253) demonstrated in dogs that the sphincter mechanism in cardia is not due to the diaphragm but to the cardia itself. Whether there is a real sphincter could not be judged.

Fyke, Code & Schlegel (254) in healthy people found a region with increased pressure from 1 to 2 cm. below to 1 to 2 cm. above the hiatus diaphragmatica. This physiological sphincter "relaxes" during swallowing.

Stomach and intestine.—Iggo (255) studied the electrical activity in single afferent C-fibers of the vagus originating from the oesophagus, stomach and intestine. Evidence for stretch receptors connected in series with the contractile elements was found.

After food the motility in the antrum was mostly decreased (256) but the idea that morphine should delay the emptying by increasing the tonus of the

pyloric region was not confirmed [Crone & Ardran (257)]. Lorber & Shay (258) studied the influence of changes of pH and osmolarity and of fat in the duodenum on the motor activity of the pylorus. Hunt continued his work on the emptying rate of the stomach (259) and related it to precardial osmoreceptors. His observations on the passage of different salt solutions are interesting but his conclusions about osmoreceptors and their permeability properties need more evidence to be conclusive.

Insulin is a good stimulant for motility and propulsion when studying musculotropic agents according to Lish & Peters (260). Glucagon inhibits the motility of the stomach and colon for 30 to 45 min. (261).

Muren (262) found three phases in motility of the stomach after vagotomy: decreased during the first days; increased in the following two to three months and a successive decrease in later periods. After vagotomy (263, 264) or pharmacological denervation with piperidino-ethyl-diphenylacetamide (265) he found an increased sensitivity to epinephrine or norepinephrine. See also (266).

Gamma-radiation must be used in superlethal doses in order to inhibit the gastric motility in dogs according to Conrad (267), who reports that this is in contrast to the case of smaller animals. French's & Wall's (268) findings were similar when they observed that cholinesterase was inhibited in rats and guinea pigs but not in monkeys by total body x-irradiation.

Intestine.—Ritter (269) reported that there is no direct proportion between pressure and volume increase in the intestine. In duodenum the volume increases more slowly than the pressure but in the colon the opposite is true. In heavy loading the duodenum relaxes but the colon contracts. Kosterlitz *et al.* (270) discovered that low pressures in the isolated ileum increased the longitudinal tension whereas higher pressures were required to get circular contractions. The longitudinal contraction was, however, no trigger mechanism for the circular one.

Streeten *et al.* (271) concluded that the effect of the adrenals on the intestinal motility is due to aldosterone. Whether this acts through influences on the sodium/potassium ratio in the intestinal muscle is not clear. Rand (272), however, reports that a pretreatment of the isolated intestine from guinea pigs with potassium solutions increases the sensitivity to histamine, whereas acetylcholine pretreatment, that possibly deprives the intestine of potassium, decreased that same sensitivity. For further discussion on the effect of potassium on the intestinal motility see (273, 274). The rhythmicity of the isolated duodenum varied directly with pH but was not influenced by the CO₂-tension [Adelman (275)].

The nervous mechanisms in motility were investigated by Blair & Clark (276), who found that substance P is probably not a neurohormone and by Milton & Smith (277) who found evidences for a pacemaking area round the entrance of the biliary duct. Semba (278) could inhibit the motility of the colon in dogs by the stimulation of the ventral roots in spinal nerves L₂ to

L₇, but found a motor effect on stimulation of the dorsal roots. The position of the synapses for the two systems were analyzed. The same author (279) studied the nervous connections that caused an increased motor activity in the stomach but an inhibition in the intestine upon suddenly increased pressure in the urinary bladder. Stolze (280) found the "Cannon-Boehmsch point," i.e., where the short wave type of movements in the colon disappears and where the long wave type appears, to lie in the distal part of the ascending colon or in the beginning of the right flexura coli.

There is no doubt that the field of gastric and intestinal motility is very confusing and most papers contribute little to its elucidation. Therefore it is encouraging to see fresh attacks on the problem. Greven (281) found the propagation speed to be 1.0–4.0 cm./sec. both in the longitudinal and transverse direction of the gut. The propagation can proceed in any direction but probably, due to the intramural nervous plexus, some directions are preferred thus leading to a coordination. The work of Brune & Kotowski (282) also showed some fundamental facts, namely that on short distances (about 3 mm.) there is a musculo-muscular transmission with a propagation speed of 4 mm./sec. and with acetylcholine as transmitter substance.

The effect of local anaesthetics (283), muscular activity, hyper- and hypothermia and electric shocks (284) on the intestinal motility were also studied, as well as the speed of passage through the intestine of the goat (285).

Attempts to study the innervation of the sphincter of Oddi did not give consistent result (286) nor did the experiments on the electrical excitability of the human gall bladder with an otherwise good technique (287).

Bishop *et al.* (288) made electromyographic records from the external sphincter of the anus in decerebrated cats. Inhibiting as well as augmenting impulses from the surroundings and colon seem to enter through the pudendal nerves.

THE ULCER PROBLEM

The reviewer has not particularly studied this part of digestion but even in the field of physiology a few papers should be mentioned. First the work of Barrett (289), who was interested in possible genetic factors. Accumulation of gastric juice in the stomach or injection of gastric juice from rats in the same strain produced very few ulcers in fasted rats. Injection of juice from rats of other strains gave a high incidence of ulcers. The work unfortunately suffers from the low numbers of controls, but more work along the line is urgently needed. Dillard & Merendino (290) showed that the different parts of the digestive tract have equal resistance to gastric juice [c.f. (111)—pepsin].

Sundell & Teir (291) found that if a vagotomy preceded the pyloric ligation in the Shay rat the incidence of ulcer was much greater.

MISCELLANEOUS

Brooks, McCann & Brobeck (292) described an operation in dogs that provides a good aid in teaching gastroenterology.

The *post mortem* content in the digestive tract of sheep was analyzed (293) and the effect of some different diets on the dry weights of different parts of the canal determined (294). Dobson (295) studied the blood and lymphatic supply to the rumen epithelium of sheep.

Welbourn & Doggart (296) showed that the secretory function of the rat stomach is much more important in the nutrition of the animal than its storage function.

Hartiala *et al.* (297) discovered that the synthesis of glucuronide in the liver but not in the duodenum increases after thyroxin administration in rabbits. After injection of radioiodine in rats the opposite happens (298). Buckeridge & Freeman (299) found that the lactone form of glucuronic acid is absorbed much more rapidly in the intestine than is the salt form. Schalm *et al.* (300) demonstrated the interesting facts that an increment of bilirubin in blood does not occur after partial obstruction of the bile ducts. Only when a complete stop exists will icterus develop. Occlusion of part of the liver in the bile duct or portal vein results in an atrophy of the occluded part and a hypertrophy in the rest.

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BLOOD (FORMED ELEMENTS)^{1,2}

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Five years have elapsed since this subject was last dealt with in the *Annual Review of Physiology* (1). In reviewing elsewhere the hematological literature for the single year of 1953, Wright *et al.* (2) discussed 1,036 papers written in English and Begemann & Sievers (3) 472 written in German. Since then the rate of publication throughout the world has greatly increased. Without further explanations or apologies the reviewer will now, in the limited space allotted to him, discuss or mention 340 papers which, with rare exceptions, were published between June, 1952 and June, 1957. His selection, made arbitrarily from a world literature many times as great, will illustrate rather than adequately document a few of the important advances of that period.

METHODS

Separation of formed elements.—An improved form of the Cohn blood fractionator, described in detail by Tullis *et al.* (4), has been developed and made commercially available. In it blood flows continuously and aseptically from a vein of the donor through an inverted cone centrifuge, its various fractions passing without exposure to the outside air into appropriate sterile containers. Removal of calcium by an ion exchange resin prevents coagulation. A modification of the apparatus permits the plasma to be retained in a plastic bag while the formed elements, resuspended in a physiological salt solution, are returned to the vein from which they issued. In a recent series of plasmaphereses by this method, Smolens *et al.* (5) report an average time from body to body, for 500 ml. of blood, of 22 min. The regeneration of the plasma proteins, including potent antibodies, is so rapid that the same donor has submitted to bi-weekly plasmaphereses for a period of 40 weeks (since further extended) with no evident harm. The apparatus can also, without osmotic injury to the erythrocytes, remove high concentrations of glycerol added to permit their preservation by freezing. In the ordinary centrifuge reticulocytes rise to the top of the red cell column; Borun *et al.* (6) have shown by Fe⁵⁹ labeling that there is a further stratification according to age among the mature erythrocytes, and that a method is thereby provided for the estimation of their total life-span. Keitel *et al.* (7) have studied chemical differences within the column of packed cells, and Chaplin & Mollison (8) have measured the relation of the plasma error of the hematocrit to the

¹ The survey of literature pertaining to this review was completed in June, 1957.

² The following abbreviations are used in this chapter: AMP, adenosine monophosphate, ACDI, acid citrate dextrose inosine, PNH, paroxysmal nocturnal hemoglobinuria, ACTH, adrenocorticotrophic hormone.

length of the cell column. Conversion factors for calculating the theoretical body hematocrit from the observed venous hematocrit have been determined by Reeve *et al.* (9) and by Chaplin *et al.* (10). For a better separation of leukocytes and platelets from erythrocytes Coxon & Robinson (11) and Maupin & Chary (12) have employed special types of centrifuge vessels, while Skoog & Beck (13) have accelerated the separation of the erythrocytes with dextran, fibrinogen, and a phytoagglutinin. By supplementing plasma with two albumin solutions of different densities, Agranoff *et al.* (14) have been able simultaneously to separate lymphocytes, polymorphonuclear leukocytes, and erythrocytes with a purity in each case of more than 90 per cent. Wilson (15) has found that an isosmotic solution of NH_4Cl removes contaminating erythrocytes from a suspension of leukocytes without injury to the latter.

Electron microscopy.—Particularly noteworthy in this field are the results of Hillier & Hoffman and their associates (16, 17, 18). Using a resolving power higher than most of their predecessors and obtaining virtually complete removal of hemoglobin by the method of successive hemolysis, they found the erythrocyte membrane to have an orderly structure of considerable complexity (16), reproducible for the cells of a single individual (17), somewhat abnormal in certain types of anemia (18), and showing characteristic differences in different species of mammals (17). More specifically (16) the surface of the human erythrocyte is covered by a layer of circular plaque-like structures approximately 100 to 500 Å in diameter and 30 Å in thickness, the total number per cell being 10,000 or more. In the groundhog they are much larger; in some other species, smaller. They are removed without destruction by an alcohol-ether-chloroform mixture, disclosing the presence of an underlying layer of protein fibers, and perhaps represent the elinin fraction of Moskowitz & Calvin (204). A structure of this general nature appears indistinctly in photographs by a number of recent workers, but very clearly in one obtained by Coman & Anderson (19) by a chromium-carbon replica method. These findings are of much theoretical interest in that the interstices among the plaques seem to provide the aqueous channels long postulated to explain the exceptional permeability of erythrocytes in general to water and anions, and of those of the groundhog to hydrophilic nonelectrolytes of high molecular volume [see Jacobs (254)]. Other references to electron microscopy, particularly by the method of thin sectioning will be found below, e.g., (114, 142, 144, 145, 146, 271, 279).

x-Irradiation.—This subject has been dealt with very thoroughly in a recent review by Bond & Cronkite (20) and will not be discussed here except for a single illustration of its growing importance as a tool in hematology. Appropriate x-irradiation of the body as a whole simultaneously damages its hematopoietic tissues and depresses its immunological defenses. Employing this technique, Jacobson *et al.* (21) showed that lethally irradiated mice could be protected by the subsequent administration of splenic tissue intraperitoneally, while Lorenz *et al.* (22) obtained the same result with bone

marrow cells intravenously. For a time there was a difference of opinion as to whether the protection was a result of a repopulation of the hematopoietic organs by normal cells or of a favorable effect on them of a humoral agent of some sort, the evidence at first favoring the latter view. In 1954 Barnes & Loutit (23) criticized this evidence and offered some of an immunological nature in support of the cellular theory. In the same year, Congdon & Lorenz (24) obtained protection in the mouse by bone marrow from the rat, thereby providing a convincing method for the identification of transplanted cells. Within a short time Nowell *et al.* (25, 26) by alkaline phosphatase reactions of leukocytes and Makinodan (27) by the use of antirat and anti-mouse sera had demonstrated a survival of functional rat cells in the mouse. Even earlier, Lindsley *et al.* (28), using rats whose erythrocytes could be identified by an agglutination reaction, had produced persistent erythrocyte mosaicism in that species. Ford *et al.* (29), by the use of a chromosome marker, also demonstrated the survival of cells of one mouse in the body of another. The possibility of both destroying and replacing bone marrow cells at will led to the findings of Russell *et al.* (30) that in a certain type of hereditary anemia in mice characterized by erythrocytes of large size, irradiation followed by introduction of normal marrow produced a lasting rise of the hematocrit and a reduction of the cell size. By similar treatment Barnes *et al.* (31) produced a 70 per cent three month survival of leukemic mice, nearly all of the controls dying within a few weeks. Miller (32) has obtained protection by tissue culture cells, and Barnes & Loutit (33) by glycerol-treated cells frozen at -79°C . for several months.

Isotopic labeling.—The isotopes Cr^{51} , Fe^{59} and Co^{60} have been chosen for special mention because each forms the basis of an important method that has come into widespread use during the past five years. Gray & Sterling (34) first showed the practicability and advantages of using Cr^{51} as a label for human red cells. Unlike iron, chromium can be promptly incorporated *in vitro* in a sample of the subject's own erythrocytes, and in the body it causes no confusion by re-utilization. By this method, Sterling & Gray (35) measured the total red cell volume in man. Ebaugh *et al.* (36) further standardized the method and used it to determine normal survival curves. Necheles *et al.* (37) did the same for normal and Weinstein & LeRoy (38) for abnormal erythrocytes. With the work of Read *et al.* (39) and Sutherland *et al.* (40) in 1954 the method had become well established and was yielding results of value in both health and disease. Its status at that time and some of its general theoretical aspects were discussed by Mollison & Veall (41) and by Donohue *et al.* (42). Since then, publications in the field have become far too numerous for more than illustrative examples of a few recent special applications of the method. Gibson & Scheitlin (43) used it to investigate the viability of stored erythrocytes; Mollison & Cutbush (44), the compatibility of blood for transfusion when the usual typing methods fail; Pritchard & Weisman (45), the uptake of red cells from the peritoneal cavity; Owen *et al.* (46) and Roche *et al.* (47), gastro-intestinal blood losses;

Jandl *et al.* (48) the sites of sequestration of erythrocytes; Crawford & Mollison (79), the purity of unagglutinated red cells; Hollingsworth & Hollingsworth (49) sex differences; and Jandl *et al.* (48) splenic destruction of red cells and the desirability of splenectomy.

In 1950 Huff *et al.* (50) had found that a small amount of Fe^{59} introduced into the human circulation disappears approximately exponentially. Assuming this relation to be generally true, they set up equations for calculating turnovers of iron in the plasma and red cells from measurements which at least in principle are simple. For normal human subjects they calculated an average daily replacement of 0.85 per cent of the erythrocyte iron. Later papers dealt with the effects of altitude on erythropoiesis (51), polycythemia produced by transfusion (52), and the distribution of Fe^{59} in the body as determined by surface measurements of radioactivity over the spleen, liver, heart, and sacrum, respectively (53). Wasserman *et al.* (54) also used the removal of Fe^{59} from the blood as an index of erythropoiesis. The method has recently formed the basis of systematic studies in the field of "erythrokinetics" by Giblett *et al.* (55) on normal and anemic blood, Finch *et al.* (56) on pernicious anemia, Sturgeon & Finch (57) on Cooley's anemia and Bothwell *et al.* (58) on various types of hyper- and hypofunction of the bone marrow. Bothwell *et al.* (59) have also contributed a general discussion of the method itself. Another very fruitful use of the principle of iron utilization has been for the assay of erythropoietin by Jacobson and his associates (98, 99, 100, etc.), Mirand & Prentice (101, 102) and others. A further refinement by Weinstein & Beutler (60) is the combination of Fe^{59} and Cr^{51} to permit blood formation and destruction to be measured simultaneously.

Radioactive cobalt is now being widely employed in problems of erythropoiesis where vitamin B_{12} (which contains cobalt) and Castle's intrinsic factor (which promotes the intestinal intake of B_{12}) are involved. For a general background of this field Castle (61), and for many references to the recent literature Ungley & Thompson (62) may be consulted. In 1950 Chaiet *et al.* (63) by a biosynthetic method incorporated Co^{60} in B_{12} . Heinle *et al.* (64) then showed that $\text{Co}^{60}\text{B}_{12}$ administered by mouth in pernicious anemia was mostly eliminated in the feces; the loss however could be greatly reduced by the simultaneous administration of the intrinsic factor. This observation, (since abundantly confirmed) and essentially the same result obtained by Swendseid *et al.* (65) following total gastrectomy, have greatly clarified the role of the gastric factor. Similar principles govern the elimination of $\text{Co}^{60}\text{B}_{12}$ in the urine in a manner and under conditions first investigated by Schilling (66) and now the basis of a valuable diagnostic test for pernicious anemia [see further Rath *et al.* (67)] It is also possible to follow the uptake of $\text{Co}^{60}\text{B}_{12}$ by external scintillation counting over the liver [Glass *et al.* (68)] and by direct observations of the blood [Doscherholmen & Hagen (69)]. To reduce dangers due to the long half life of Co^{60} (5 yr.), Bradley *et al.* (70) have recommended, and they and others have since used, Co^{58} with a half life of 75 days. The finding of Whipple *et al.* (71) that $\text{Co}^{60}\text{B}_{12}$

administered during blood regeneration appears temporarily in the stromata but not in the hemoglobin of newly formed erythrocytes tends to confirm the view that the primary function of this vitamin in erythropoiesis is connected with stroma formation.

Preservation by freezing.—Following the observation by Smith (72) that red cells frozen at -79°C . in the presence of 15 per cent glycerol preserve their normal appearance when thawed, Sloviter (73) showed that rabbit erythrocytes so treated survive when transfused into another rabbit. Blanchaer & Mayman (74) found that the glycolytic powers of red cells were still intact after four months at -79°C . Human transfusion with cells preserved in this way was accomplished by Mollison & Sloviter (75) followed by Brown & Hardin (76), and has since been repeated many times. Chaplin *et al.* (77) have indeed reported a 24 hr. post-transfusion survival of 82 per cent after storage for 21 months. Since the temperature of -79°C . obtained with solid CO_2 is troublesome to maintain, Chaplin *et al.* (78) have experimented with one of -20°C . in an ordinary "deep freeze" and found it usable though inferior to the lower one. Erythrocytes at -20°C . show appreciable evidences of metabolism and, according to Crawford & Mollison (79), lose most of their potassium over a period of several months, though they can regain it within four days after transfusion into a suitable recipient. Erythrocytes for use in blood typing can be preserved in satisfactory condition for at least a year at either temperature [Crawford *et al.* (80); Grove-Rasmussen *et al.* (81)]. Carrying the temperature in the opposite direction, Meryman & Kafig (82) obtained post-transfusion survival of human erythrocytes that had been sprayed into liquid nitrogen at -196°C . It is often thought that the injury from which the glycerol protects the cells is due to mechanical effects of ice crystals, but Lovelock (83, 84, 85) has given reasons for believing it to be associated with the high electrolyte concentrations resulting from the removal of liquid water from the system. Glauser & Talbot (86) find evidence for both types of injury. From a practical point of view one of the greatest difficulties of the method is the removal of the glycerol from the cells without causing their osmotic hemolysis. Among the methods that have been employed are dialysis (75), washing in solutions of decreasing glycerol concentration (73), logarithmic addition of a physiological salt solution (74), the use of the Cohn fractionator (4) and allowing the glycerol to diffuse into a hypertonic solution of sodium citrate (87) or sodium lactate (76). Sloviter (88) has recently suggested a simplified technique by which the thawed blood is merely mixed with concentrated glucose, after which physiological NaCl can safely be added.

ERYTHROCYTES

Control of erythropoiesis.—In 1952 Grant & Root (89) summarized the evidence that had recently made the old theory of oxygen tension in the bone marrow untenable. Belief in a humoral agent produced elsewhere in the body was then beginning to seem reasonable but, except for the findings

of Reissmann (90) with parabiotic rats, it had little experimental support. This soon became available. Erslev (91), using much larger quantities of anemic rabbit blood than previous workers, produced in other rabbits consistent rises in total red cell and reticulocyte counts, with microscopic evidence of increased activity of the bone marrow. Stohlman *et al.* (92) in a patient with a patent ductus arteriosus found that hypoxia in another part of the body could increase the activity of normal bone marrow. Other indirect evidence of this sort is mentioned by Erslev (93). Successful attempts to obtain from the blood of anemic rabbits extracts that stimulated erythropoiesis in normal rats were reported by Borsook *et al.* (94) and Gordon *et al.* (95). Later work in this field is described by Linman & Bethell (96). Piliero *et al.* (97) obtained similar extracts from human anemic blood.

The most convincing evidence for the existence of erythropoietin (an older term now coming into general use) has been made possible by the method of iron incorporation, already described. The standard assay method of Jacobson and his associates (98, 99, 100) takes advantage of the high sensitivity of hypophysectomized rats to erythropoietin [see also Mirand & Prentice (101, 102)]. From the results of their most recent work Jacobson *et al.* (103, 104, 105, 106) have deduced an important principle, namely, that the production of erythropoietin is regulated not by the supply of oxygen to the body, as such, but by the relation between oxygen supply and demand. In a search for the site of production of erythropoietin Gordon *et al.* (107) obtained negative results with extracts of tissues other than the blood. Fried *et al.* (103), using hypophysectomized animals both for the test and the assay, showed that the hypophysis itself is not necessary; they also stated without experimental details, that erythropoietin production is not prevented by splenectomy, thyroidectomy, adrenalectomy, or gonadectomy and this list was later extended (106) to include the stomach, intestine, pancreas and 7/8 of the liver. Very recently, however, by ablation experiments, Jacobson *et al.* (106) have obtained evidence that the kidney may be the organ chiefly involved, presumably through an activity other than excretion, since tying both ureters did not abolish the response to hypoxia.

It is impracticable to enter here into the much disputed question of the relation of the known endocrine organs to erythropoiesis. Many references to the literature are given by Van Dyke *et al.* (108) and by Gordon (109). The chief disagreement centers around the hypophysis. On the one hand, Jacobson and his associates, in the light of the principle mentioned above, emphasize its indirect effect through its influence on the metabolism of the body as a whole. In their latest paper, Crafts & Meineke (110) adopt a similar view, which is not inconsistent with their previous findings (111) concerning thyroid and adrenal influences. On the other hand, Van Dyke *et al.* (112) have recently strongly reaffirmed their belief that in addition to the erythropoietin so far discussed, for the production of which they agree that the hypophysis is not necessary, there is another of pituitary origin. The latter is said to resemble but not to be identical with ACTH, from which they have been able partially to separate it.

Aging and life span.—The aging process begins with the loss of the special characteristics of the reticulocyte which are discussed at length, with several hundred references to the literature, by Seip (113). Chalfin (114) in a systematic comparison of the reticulocyte with the mature erythrocyte gives further information concerning morphology, physical properties, K:Na ratio, etc. His electron microphotographs of reticulocyte sections show typical mitochondria with laminated membranes; these structures also appear in similar photographs by Braunsteiner *et al.* (115). The metabolism of the reticulocyte has been investigated by Sherwood Jones (116) who finds evidences of both the Krebs cycle and the hexose monophosphate shunt. Rapoport *et al.* in a series of papers, to the others of which the latest one (117) gives references, have reported that the cessation of oxidative metabolism is brought about by a succinic oxidase inhibitor which appears, produces its effects and then itself disappears. Kruh & Borsook (118) have found that reticulocytes utilize labeled amino acids at about the same rates for the synthesis of heme and of globin, which strongly suggests that hemoglobin is being synthesized. The activities of twelve enzymes were studied during the period of transition by Rubinstein *et al.* (119); five showed a slight or moderate decrease, three a very great decrease and four a complete disappearance. Allison & Burn (120) followed three other enzymes through the entire life of the red cell and found what appeared to be an exponential decline in their activity. According to Beutler *et al.* (172) hemolysis *in vivo* by primaquine depends upon the age of the cells. By labeling the latter with Fe^{59} during their formation in a susceptible subject, they found that when the labeled cells were young (8 to 21 days) they were spared; later (63 to 75 days) they were preferentially destroyed. The metabolic background of this finding is discussed below (173, 174). Erythrocytes do not however always show evident deterioration with time; Gabrio *et al.* (156) reported that their ability to tolerate storage *in vitro* is unrelated to their *in vivo* age.

Rodnan *et al.* (121) measured the life spans of the erythrocytes of the chicken, duck and pigeon, and combining these with data from the literature for eight species of mammals and the turtle, constructed a table, very useful for reference. The reciprocals of the values so determined show a good correlation with the basal heat productions of the species in question. In the marmot Brace (122) found the life-span to be greatly lengthened during hibernation and even in a cold environment in the absence of hibernation. Brown & Eadie (123) discussed the factors and mathematical principles involved in measurements of life-spans, and contributed new data for several species. Eadie & Brown (124) determined normal human life-spans simultaneously with Cr^{51} as a label and with an anti-A hemolysin in place of the usual agglutinin. The values so obtained agree well with each other and with those in the literature. A new label employed by Cohen & Warringa (125) likewise gave a value in good agreement with the generally accepted one. Evans (126) however, has called attention to the presence in normal persons of some very short-lived erythrocytes, and Hollingsworth (127) has found a relatively short life-span in fetal red cells introduced into the adult circula-

tion. According to Van Dyke *et al.* (128) the life-span of rat erythrocytes is not lengthened by cobalt administration; the same was found to be true of the effects of low barometric pressure for rats by Fryers & Berlin (129) and for men by Berlin *et al.* (130). The cause of the polycythemia in such conditions will therefore continue to be sought elsewhere, as it has been in the past. Schrumpf (131) found that spherocytes which had a survival period of 10 days when introduced into the circulation of a normal person of an appropriate blood group had one of about 100 days in a splenectomized but otherwise normal individual.

Form and structure.—The biconcave form of the erythrocyte is obviously useful in permitting moderate volume changes without any stretching of the delicate cell membrane. Its frequently emphasized importance for rapid gas exchanges has been questioned by Roughton (132) whose calculations, taking into account factors other than simple diffusion, indicate little advantage of a biconcave over a spherical form. Recent experiments by Carlsen & Comroe (133) tend to confirm this view. In studies of reversible disc-sphere transformations, Trotter (134) showed the importance of mere traces of contaminating lipid-like substances from the human skin; Brown (135) cast doubt upon the view that the normal shape of the erythrocyte depends upon a layer of anti-sphering protein at its surface; Barer & Gaffney (136) showed that under conditions where the plasma of several mammals, including man, has a typical anti-sphering effect, that of rats and mice causes sphering; Hamilton & Ravdin (137) followed the development of a sphering agent in plasma *in vitro*, connecting with it the poor survival of dog erythrocytes in plasma-containing media; Pranker *et al.* (165, 166) suggested that the form of the erythrocyte depends upon a contractile substance (adenosinetriphosphatase?) at its surface, the failure of which under abnormal metabolic conditions might cause spherocytosis.

The structure of the erythrocyte has been very thoroughly discussed by Ponder (138, 139) and by Bessis (140). Among the major points of disagreement at present is the thickness of the cell membrane. Five years ago this was rather generally believed to be of the order of 100 to 200 Å, but more recent estimates range from 50 to 5,000 Å. Some of those made since 1952 and the methods used to obtain them are as follows: Parpart & Ballentine (141), chemical analysis, 52 Å; Hillier & Hoffman (16), electron microscope with shadowing, 50 Å; Chalfin (114), electron microscope with thin sections, 60 to 70 Å; Bernhard *et al.* (142), electron microscope with thin sections, 60 Å; Ruhenstroth-Bauer & Schmidt (143), optical interference, 197 Å; Bernhard (144), electron microscope with thin sections, 160 to 800 Å; Kautz & De Marsh (145), electron microscope with thin sections, 200 to 300 Å; Latta (146), electron microscope with thin sections, 140 to 1,000 Å; Mitchison (147), polarization optics, 5,000 Å for an inner protein and 40 Å for an outer lipid layer. Apart from imperfect resolution by the electron microscope and different degrees of hydration, the factor of most importance in producing such diverse results seems to be the amount of hemoglobin left in the stromata after hemolysis. This is greatest by the method of Mitchison

in which hemolysis takes place in glycerol; according to Ponder & Barreto (148) the birefringence of ghosts so produced, as well as their visibility with the ordinary microscope is due to their hemoglobin. At the opposite extreme are the ghosts studied by Hillier & Hoffman and by Parpart & Ballentine which were virtually hemoglobin-free.

Metabolism.—Recent work on normal erythrocyte metabolism is too extensive and too chemical in nature for discussion here, but several of its more practical aspects may be mentioned. A general chemical background can be obtained from Prankerd (149, 150) and Gabrio *et al.* (151), with fuller details from Dische (152), Prankerd & Altman (165) and Bartlett *et al.* (153, 154).

In a short communication in 1952, Gabrio & Finch reported that a defect in stored erythrocytes, which they believed to be due to a deficiency of ATP, could in part be repaired by a temporary sojourn in the circulation of a living animal. Gabrio *et al.* then showed that the "storage lesion," more fully described in a longer paper (155), is not related to the *in vivo* age of the erythrocytes (156) and is little influenced by the exact conditions of storage other than its duration (157). Following earlier observations of Dische (152), they and Prankerd *et al.* independently began to use adenosine to remedy metabolic deficiencies. With this substance it was possible to reverse the "storage lesion" *in vitro* (158), the treated cells regenerating organic phosphates, recovering lost potassium, and showing improved post-transfusion survival. (159). Other purine nucleosides can replace adenosine, inosine being particularly effective, and an ACDI² storage solution containing the latter substance was found to give excellent results (160, 161). Hennessey *et al.* (162) give further information concerning the metabolic degradation of nucleosides *in vitro*, and Gabrio *et al.* (151) and Donohue *et al.* (163) discuss the metabolic aspects of blood storage. From a different point of view, the independent work of Prankerd *et al.* (164, 165, 166, 167, 168) has contributed important information about the manner of entrance of the constituents of the nucleosides into the erythrocyte, their fate in the latter, their usefulness in correcting the metabolic deficiencies of hereditary spherocytosis, and many other points of interest. Rubinstein *et al.* (169, 170) have also obtained results in general agreement with those mentioned. Very recently Overgaard-Hansen *et al.* (171) found that erythrocytes in a glucose-free medium, when supplied with inosine, could convert most of their AMP and ADP to ATP within seven minutes.²

The hemolytic effect of primaquine *in vivo* (172) has been further studied by Beutler *et al.* (173), who find it to be associated with an age-dependent loss of reduced glutathione. Drug sensitive cells are affected similarly by acetylphenylhydrazine and, unlike normal cells, cannot be fully protected by glucose or inosine. These results are believed to be consistent with the suggestion of Carson *et al.* (174) that the metabolic defect is a decreased activity of glucose-6-phosphate dehydrogenase. A more common type of hemolysis *in vivo*, which seems likewise to be associated with a metabolic defect, is found in spherocytic hereditary hemolytic anemia [for a recent

full discussion see (175)]. According to Prankerd *et al.* (166), in this condition, the internal specific activity of P^{32} supplied to the cells becomes higher in the orthophosphate than in the organic phosphates, which is the reverse of the distribution normally observed and which in their opinion indicates a defect in phosphorylation. A very similar view, based on similar evidence, was also expressed by Motulsky *et al.* (176). Both groups of workers found that adenosine could reverse the defect.

Another metabolic abnormality of the erythrocyte not connected with hemolysis, and shared with other cells of the body, is its inability in certain persons to metabolize galactose [Schwarz *et al.* (177)]. A test of the erythrocytes of a galactosemic individual may indeed be used as a diagnostic procedure. Further studies of such erythrocytes by Kalckar (178) showed a defect in the functioning of the enzyme galactose-1-phosphate transferase.

Much interest has been aroused by the suggestion of Blowers & Maizels (179) that a peculiar flickering appearance, best seen with the phase contrast microscope, is visible evidence of metabolism within the erythrocyte. Parpart & Hoffman (180), however, found the same appearance in erythrocytes fixed with formaldehyde or autoclaved. Their belief that the effect is caused by minute Brownian movements of the cells is supported by its disappearance in media of high viscosity.

Hemolysis.—Recent work seems to indicate that osmotic hemolysis involves a state of leakiness, without rupture of the erythrocyte membrane, due to stretching when the volume of the cell exceeds a certain critical limit. Under favorable conditions escape of hemoglobin and other cell constituents may then relieve the strain and permit the membrane to resume its original area and other normal properties. A careful study of osmotic ghosts by Teorell (181) showed them to be excellent osmometers. Stein (182) also obtained good quantitative agreement in the measured rates of entrance of a series of nonelectrolytes into human erythrocytes and their osmotic ghosts, the similarity extending even to the effects of copper on glycerol permeability. Studies of the stage of temporary leakiness by Székely *et al.* (183) showed that at the time of escape of hemoglobin there was also an abnormal permeability to ions such as K and PO_4 . Straub used this principle to obtain sodium-containing "reverted ghosts" for his experiments on potassium transport mentioned below (216). Osmotic ghosts prepared by a single exposure to a hypotonic solution typically contain some hemoglobin resistant to removal by washing and therefore frequently thought to be combined with the stroma in a non-diffusible form—for which according to Ponder *et al.* (138, 184) there is also other evidence. The fact remains, however, that Hillier & Hoffman (16) were able to remove virtually all the hemoglobin from their ghosts, not by three successive washings but by three successive periods of leakiness. Hoffman (185) also availed himself of the leaky period to reintroduce hemoglobin into ghosts in chosen concentrations for various experimental purposes. An aspect of osmotic hemolysis too frequently neglected in osmotic fragility measurements has been taken into account by Emerson *et al.* (186) who have constructed a useful table of fragilities cor-

rected for the osmotic effects of pH differences. Jacobs (187) has also called attention to possible errors associated with HCO_3^- and Cl^- exchanges when normal erythrocytes are placed in or washed with large volumes of physiological NaCl . Though such an exchange, in itself, is isosmotic, its indirect effects on the cell volume may be considerable.

Differences of opinion still exist concerning the mechanism of hemolysis by simple chemical compounds such as sodium cetyl sulfate and other synthetic detergents. Croes & Ruysen (188) attributed lysis by these agents to the attainment of a critical degree of adsorption at the cell surface, Pethica & Schulman (189) to the attainment of a critical surface tension involving the cell cholesterol, and Hutchinson & Bean (190) to a simple penetration of the cell lipides. L. Love (191), working with complete hemolysis curves, found that with certain concentrations of the lysin the process began rapidly, then came almost to a standstill, again to be resumed and carried to completion. She explained her results by the production of a state of cation permeability of the cell surface complicated by a "blocking" effect of the lysin itself which could be removed at will by dilution or by the addition of a barium salt. Rideal & Taylor (192) obtained hemolysis curves of the same sort but attributed the initial rapid phase, which can be prevented by previously washing the cells, to an attack by the lysin on removable lipides at the cell surface. Love (193), with a cationic detergent obtained additional evidence of the importance of induced cation permeability and of the reality of the blocking effect. In a second paper (194) he dealt quantitatively with the combination of the detergent with the cell surface. Directly connected with the problem of induced cation permeability and colloid osmotic hemolysis is a method proposed by Wilbrandt (195) for recognizing the latter. Cook (196) has used it, among other criteria, to show that the mechanism of a type of ultraviolet hemolysis which he studied is of this general nature. Related to the presence of loosely attached lipides at the cell surface is the finding of Lovelock (197) that after their removal by hypertonic salt solutions a sudden cooling of the cell causes hemolysis.

The last of an important series of papers by Mayer & Levine (198) presents in concise form a proposed scheme of immune hemolysis according to which (a) sensitization occurs (b) the sensitized erythrocyte in the presence of calcium reacts with one part of the complement complex (c) in the presence of magnesium a second part of the complement complex is added and (d) hemolysis follows. Actually, steps (b) and (c) may each involve at least two processes. A new principle was introduced when Pillemer *et al.* (199) discovered the substance in the blood which they called properdin [for a short general review see Isliker (200)]. Though properdin seems to be a part of a general nonimmune protective system of the body against foreign organisms it may on occasion take part in the destruction of abnormal red cells belonging to the body itself. A case in point is paroxysmal nocturnal hemoglobinuria. According to Hinz *et al.* (201), removal of properdin from normal serum renders it inactive for hemolysis of PNH^2 cells but not for immune hemolysis. Activity is restored by the addition of properdin. Necessary for

this type of hemolysis are magnesium and constituents of the blood indistinguishable from those of complement. Similarly, (202) after treatment of erythrocytes with tannic acid they can be hemolyzed by normal plasma, but not by plasma from which the properdin has been removed. Many papers have been published recently concerning the process of autohemolysis. Though the concept of autoimmunization is not a new one, important light was thrown on its relation to the blood group antigens by the finding by Weiner *et al.* (203) in the blood of a person suffering from acquired hemolytic anemia of red cells possessing the antigen e of the Rh system together with anti-e in the plasma. Anti-e was also found attached to the erythrocytes from which it could be eluted and tested against other e cells. Many similar cases have since been reported. For further information concerning this and related subjects Kabat (277) may be consulted.

Stromata.—Chemical analyses of stromata have been made by Parpart & Ballentine (149) and by Moskowitz & Calvin (204) who suggest possible arrangements of their various constituents. Changes in chemical composition in several anemic conditions have been studied by Tishkoff *et al.* (205). Turner (206) has found by chromatography that lecithin, though present in the other stromata he studied, is lacking in those of the ox, sheep, and goat, all of which are highly resistant to hemolysis by cobra venom. Included in the stroma are a number of enzymes; of these, an ATPase of as yet unknown function has been investigated by Garzó (207), Clarkson & Maizels (208), and Herbert (209). A cholinesterase is also present but, as Wilde (210), Harris (211), and Prankerd (150) have all pointed out, the role formerly ascribed to it in ionic transport seems questionable. Additional unfavorable evidence is the finding by Parpart & Hoffman (212) that supposed effects of the enzyme can be produced by dilute acetic acid [see also Holland & Graham (213) for effects of acidity] and an observation by Gárdos (214) mentioned below. Under favorable conditions stromata may show both metabolism and ionic transport. Székely *et al.* (215) found that following mild osmotic hemolysis, ATP metabolism continued with little diminution. Straub (216) showed that with the assistance of ATP, "reverted sodium stromata" can accumulate K against concentration gradients. Since they and normal erythrocytes are both impermeable to ATP, as such, the latter was supplied by an indirect method; but Gárdos (214) later developed a procedure by which it can be introduced directly in high concentrations at the time of hemolysis. He also found that reverted ghosts may continue their ionic transport after complete inactivation of their cholinesterase. Lionetti *et al.* (217) in further studies of stromata found that the external addition of adenosine and phosphate caused rapid movement of the latter into morphologically intact ghosts as well as into normal red blood cells. Movements of K in the two cases were comparable in rate, as was also the magnitude of the pentose metabolism. The ghosts were, however, unable to metabolize measurable quantities of glucose, and the significant amounts of lactic acid they produced were presumably derived from the pentose of adenosine.

Hemoglobin.—Few new subjects of such a specialized nature have been so

thoroughly reviewed as the abnormal varieties of human hemoglobin, most of which have been discovered since 1952. For full information, Itano (218, 219, 220), Neel (221, 222), Zuelzer *et al.* (223), and White & Beaven (224) may be consulted. One point of special biological interest concerns hemoglobin S, the molecular basis of sickle cell anemia. This disease is a serious one inherited in Mendelian fashion; why then has it not been eliminated by natural selection? Allison (225) suggested that inasmuch as it is characteristic of races living in malarial regions, the peculiarities of the hemoglobin of the heterozygotes (in whom sickling and severe anemia do not usually occur) might conceivably reduce their susceptibility to attack by the malaria parasite. In support of this hypothesis he found (226) by actual inoculation experiments a higher percentage of "takes" in normal persons than in sickle cell heterozygotes.

Important additions have been made to the comprehensive program of research of Roughton and his associates on oxygen transport, as related to the intermediate compound hypothesis. Roughton (227), Roughton *et al.* (228), Gibson & Roughton (229, 230), Gibson (231), and Gibson *et al.* (232, 233). Greatly increased accuracy in measurements of dissociation curves for oxygen (228, 229, 232), carbon monoxide (227, 232, 233), and nitric oxide (230), and the introduction of the method of stopped flow (229, 231) for studying rapid reactions and flash photolysis (231) for reversing the combination of CO with hemoglobin have resulted in accurate determinations of many of the velocity and equilibrium constants involved in the theory and in placing the latter on a firm foundation. Further evidence of four successive stages in the uptake of CO by hemoglobin has been provided by Itano & Robinson (234) who used a combination of moving boundary electrophoresis and oxidation with ferricyanide to demonstrate intermediate compounds. Illustrative of the variety of other recent work on hemoglobin are papers by Ruud (235) on its absence in certain Antarctic fishes, Hawkins *et al.* (236) on its variations with age and sex, Crosby & Akeroyd (237) on its rate of synthesis, Bragg (238) on the shape and size of its molecule, Keilin (239) on the position of its hemes, Riggs & Wolbach (240) on its sulfhydryl groups, Bateman *et al.* (241) on its packing in the erythrocyte and Ponder (242) on its physical properties and sickling.

Transfer of solutes.—This subject has been dealt with in these reviews three times in the past five years, and the reader is therefore referred to Ussing (243), Wilde (210) and Harris (211) for information about all except a few recent papers. Glynn (244) has made a detailed study of Na and K fluxes under a variety of conditions. Each ion shows a passive flux in each direction, the inward one predominating for Na and the outward one for K; but neither is a simple free diffusion. There are also active fluxes of K inward and Na outward which require energy and are linked—with evidence of a one-to-one exchange. The rates of both active processes depend upon the external K concentration and cease when it becomes zero. A recent paper by Solomon *et al.* (245) on the inhibition of K transport by cardiac glycosides gives evidence that the latter compete with K for a common substrate on

the cell surface. From the kinetics of the process the affinities of nine glycosides and aglycones have been expressed as association coefficients, and other interesting calculations have been made. An earlier estimate of the part of the available glycolytic energy needed for K transport has been reduced to seven per cent, and a formerly postulated active process for efflux of K has been abandoned as unnecessary. In searching for possible carriers for Na and K across the erythrocyte surface Solomon *et al.* (246) extracted from plasma a heterogeneous material, probably lipid in nature, that can combine with Na and K, but more readily with the latter, to form complexes that are soluble in lipid solvents and dissociate in water. Kirschner (247) has also found that a definite compound, phosphatidylserine, extracted from pig erythrocytes forms lipid-soluble combinations with Na and K, but preferentially with the former.

Among the nonelectrolytes, glucose has been studied by Wilbrandt *et al.* (248) with respect to the kinetics of its entrance into and escape from human erythrocytes. Mawe (249) also, using human blood and glucose and taking special precautions to avoid injury to the cells, obtained a much better agreement with Fick's law of diffusion than had any of his predecessors. His results do not rule out a transport mechanism of some sort, but they fail to show the complexity sometimes believed to indicate its existence. Rosenberg *et al.* (250) have shown that while hydrophilic phosphoric esters of glucose do not enter erythrocytes, glucose benzoate does; and they discuss the possible relation of these results to glucose transfer. Species differences in glycerol permeability have been further investigated by Wilbrandt *et al.* (251) who give data for four new mammals and find an interesting relation between permeability and zoological classification. Jacobs (252) has called attention to a competition between ethylene glycol and glycerol for entrance into erythrocytes. The competition strongly affects the rapid process characteristic of man and some other animals, but has little influence on the slower one that either accompanies the rapid one or is alone present in many species. Bowyer (253) showed that the entrance of glucose and of glycerol into the erythrocyte probably follow different pathways. Jacobs (254) by the method of osmotic hemolysis obtained permeability coefficients for 10 nonelectrolyte with beef erythrocytes, but later (187) called attention to a possible source of error in such measurements that might cause the values to be somewhat too low, though leaving their relations to each other unchanged.

Agglutination.—The surfaces of erythrocytes normally have little affinity for proteins. The discovery by Boyden (255) that after treatment with dilute tannic acid they adsorb proteins and related substances and can then be agglutinated by appropriate antibodies has found many recent applications. Agglutination tests have been developed by Kissmeyer-Nielsen (256) for platelet antibodies, Stavitsky (257) for purified proteins, Heller *et al.* (258) for rheumatoid arthritis, Feinberg *et al.* (259) for hay fever antibodies, Price & Weiner (260) for trichinosis, Jacobs & Lunde (261) for toxoplasmosis, Scott *et al.* (262) for herpes simplex, etc., the possibilities being virtually unlimited. Brading (263) has used the method to attach group A

and group B antigens to erythrocytes of blood group O, which then become agglutinable by antibodies for A and B. Some general aspects of the action of tannic acid on the red cell surface have been reported by Edelberg (264, 265). Agglutination of erythrocytes, in the absence of tannic acid, by bacteria and viruses has become a method of practical importance which cannot be discussed here; instructive reviews with many references to the literature have been published by Burnet (266), Neter (267), and Marrack (268). Substances of plant origin to which Boyd & Shapleigh (269) have given the name "lectins," and which have specific agglutinating effects on human erythrocytes, have recently received considerable attention. Not only do they permit distinctions of various sorts among the ABO blood groups, including the subgroups A₁ and A₂, but Boyd & Shapleigh (270) have reported a lectin by which human "secretors" of any blood group can be distinguished from "nonsecretors." Electron microscope photographs by Rebuck (271) show something of the possible mechanism of agglutination of sensitized cells. Jandl & Castle (272) have found that large anisometric molecules, which normally favor reversible rouleaux formation, tend to cause irreversible aggregation of sensitized erythrocytes, and can be used as a means of identifying the latter. Papers by Wiener & Wexler (273) on the mosaic nature of erythrocyte agglutinogens and by Wiener & Gordon (274) on the relative numbers of combining sites for antibodies of different blood group systems throw further light on the molecular complexity of the erythrocyte surface.

Blood groups.—The medical aspects of this subject cannot be dealt with here, but a few points of special biological interest will be mentioned. Recent books by Race & Sanger (275) and Mourant (276) provide an abundance of general information and references to the literature in the field, while one by Kabat (277) deals particularly, and very fully, with the chemistry and immunology of the blood group antigens. Three papers by Morgan and his associates illustrate different methods of approaching the complex chemistry of the blood group substances. In the first (278) ordinary chemical analyses reveal characteristic differences in the ratios of the carbohydrate constituents of substances A₁, A₂, B, and H. In the second (279), it is shown that the breakdown of each of the substances studied, by enzymes obtained from the protozoan, *Trichomonas*, is specifically inhibited by a different carbohydrate. In the third (280), the effectiveness of known group substances and known carbohydrates in inhibiting agglutination by plant agglutinins is compared and certain chemical relationships are deduced. Many new blood types have been discovered since 1952 [for references, see Kabat (277), Levine (281), van der Hart *et al.* (282), and Eaton *et al.* (283)]. Most of the recent discoveries have involved antigens either so rare or so common that only infrequently outside the families in which they were discovered are they useful for distinctions among individuals. Caution is needed, however, in applying the terms "family" or "private" to new antigens of apparently rare occurrence. The recently discovered Diego group was first found in a family of Venezuelan Indians and was lacking in hundreds of Caucasians tested for its presence; but it has since proved to be of

widespread occurrence not only in Indians in South and North America but in Chinese and Japanese as well [see in this connection Levine *et al.* (284), Layrisse & Arends (285) and Levine & Robinson (286)].

A mixture of blood groups in one individual (chimerism or mosaicism), already known in cattle to be associated with communications between the placental circulations of twins, was found by Dunsford *et al.* (287) in a woman, genetically of type O, whose twin brother had died 25 years before; his erythropoietic cells nevertheless still continued to produce erythrocytes of type A₁ in his sister's body. Two other cases, in each of which both twins survived, were recently studied by Nicholas *et al.* (288) and by Booth *et al.* (289). Chimerism in sheep has for the first time been reported by Stormont *et al.* (290), while the condition has been artificially produced in chickens by Billingham *et al.* (291) by means of chorioallantoic grafts during the early stages of development. Filitti-Wurmser & Jacquot-Armand (292) have applied to the blood studied by Dunsford *et al.* a method based upon thermodynamic principles which indicates that although the blood in question contained cells of separate A₁ and O types, the accompanying anti-B agglutinin was of the sort normally produced by an individual having A₁O cells. Morgan & Watkins (293) raised the question: does a person of type AB carry A and B properties in separate molecules or in molecules combining both properties? Their answer favors the latter. Sneath & Sneath (294) have shown that the Lewis antigens, Le^a and Le^b, unlike those of other human blood groups, can readily be exchanged in both directions between the red cells and plasma and that a change of erythrocyte type within this group may follow a transfusion.

OTHER FORMED ELEMENTS

Lack of space forbids an adequate discussion of five years' work on all the formed elements of the blood. Inasmuch as the leukocyte was treated with special thoroughness the last time the more general subject was here reviewed (1), and the platelet was discussed in 1957 under blood coagulation, (295) it has seemed proper to devote the present article chiefly to the erythrocyte. In the scanty space that remains it must suffice merely to mention without comment a few recent papers that will illustrate several types of work on leukocytes and platelets now in progress, and will furnish further references to the literature in those fields.

Leukocytes.—Numbers and distribution: Osgood (246); cytological sex differences: Davidson & Smith (297); Kosenow, & Scupin (298), Nicholas *et al.* (288), Booth *et al.* (289); species differences: Nowell *et al.* (25, 26); electron microscopic structure: Rinehart (294), Kautz & DeMarsh (145); metabolism: Beck & Valentine (300), McKinney *et al.* (301), McKinney & Martin (302), Remmele (303), Wagner *et al.* (304), Coxon & Robinson (11); cultivation *in vitro*: Osgood & Krippaehne (305), Osgood & Brooke (306); life span: McCall *et al.* (307); extracted antibacterial substances: Hirsch (308), Skarnes & Watson (309), Fishman & Silverman (310), Fishman *et al.* (311). leukopheresis: Craddock *et al.* (312, 313); agglutination and blood groups:

Berroche *et al.* (314), Butler (315). Transfusion and cross-circulation: Brecher *et al.* (316), Hollingsworth *et al.* (317, 318). Permeability and ionic transport: Lucké *et al.* (319), Wilson (15), Hempling (320). Sources of further references: a symposium covering many aspects of leukocytic functions, with extensive bibliographies (321), Tullis (322).

Platelets.—Origin from megakaryocytes: Humphrey (323), Thiéry & Bessis (324), Albrecht (325), Izak *et al.* (326); metabolism: Campbell *et al.* (327, 328), Wagner *et al.* (304); life span: Leeksa & Cohen (329), McGovern (330); blood groups and mixed agglutination with erythrocytes: Coombs & Bedford (331), Ashhurst *et al.*, (332); absorption of serotonin: Magalini & Stefanini (333), Zucker & Borrelli (334); release of serotonin by reserpine: Shore *et al.* (335), Haverback *et al.* (336); serotonin and clot retraction: Fenichel & Seegers (337), autoimmune reactions: Harrington *et al.* (338); hemorrhagic thrombocythemia: Spaet *et al.* (339). Sources of further references are: Mann (295), Maupin (340), Tullis (322), and yearly reviews of the literature on blood coagulation by Koller in *Acta Haematologica*.

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CENTRAL AND SYNAPTIC TRANSMISSION IN THE NERVOUS SYSTEM (PHARMACOLOGICAL ASPECTS)^{1,2}

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CRITERIA FOR CHEMICAL TRANSMISSION ACROSS SYNAPSES

The swing of the pendulum, by which the theory of chemical transmission has come to assume a dominant role in concepts of central transmission, has continued. The reviewer believes that the transmission process at central nervous synapses may indeed be in principle chemical, and that the role of electrotonic potentials imposed by one neurone on another may be that of modulating the effects of specific chemical substances. But viewed pharmacologically, the evidence now accepted for central chemical transmission is often weak, certainly weaker than that put forward many years ago to support the theory of chemical transmission at neuromuscular or ganglionic synapses, when it was not generally received. The evidence for the latter junctions was essentially as follows:—that the presynaptic neurone could (a) synthesize the transmitter and (b) release it in pharmacologically identifiable form; and that its action on the postsynaptic cell should (c) reproduce the specific events of normal transmission, and (d) be antagonized by competitive blocking agents. That there should exist (e) an enzyme destroying the transmitter is also important, although the significance of its presence in the presynaptic endings is less clear than when it is found (as at the motor endplate) in close association with the postsynaptic receptor site. For rigorous proof, none of this evidence can be dispensed with; its force lies in its conjunction. Thus the placenta contains acetylcholine; cobra venom and red cells are rich in cholinesterase; and acetylcholine and drugs like it can not only excite sensory and other nerve endings but be antagonized at these sites by d-tubocurarine or hexamethonium. But at none of these sites is a cholinergic synapse concerned. Enzyme inhibitors also lead to ambiguities. Eserine, for instance, inhibits both true and pseudocholinesterase, has some atropine-like action, and can paralyze transmission in nerve (331). The phosphorous anti-esterases inhibit not only cholinesterase but proteolytic enzymes and ali-esterases (235) and may well have a concealed anaesthetic potency (313). To obtain specific effects by the use of drugs, in short, demands as much care and pharmacological insight as the use of stimulating or recording electrodes demands of electrophysiological knowledge and experience. The most decisive evidence for chemical transmission, i.e., collection and identification of the

¹ The survey of literature pertaining to this review was completed in June, 1957.

² In this chapter, subscript i or o denotes respectively intracellular or extracellular concentration of an ion.

transmitter after stimulating the pre-synaptic neurone, together with the demonstration that the transmitter applied artificially can produce the characteristic postsynaptic events, has not yet been obtained for any specific central synapse, although the work on the activation of the Renshaw cells, in conjunction with previous knowledge of chemical transmission at motor nerve endings, comes very close.

This review will be concerned chiefly with pharmacological aspects of synaptic transmission; but since pharmacological actions are being increasingly studied by electrophysiological methods, some of the work on bioelectric potentials which promises to be important for interpretation of chemical synaptic events will be discussed. Synaptic transmission at the motor endplate and at the electroplax are discussed by del Castillo & Katz (56) and by Grundfest (148) respectively. The Proceedings of the International Symposium on Curare and Curare-like Agents (178a) contain a useful account of current work on the motor endplate, including the studies by P. Waser, and C. Chagas and their collaborators on the fixation of radioactive curare preparations by endplate receptors. The symposium on Hormones, Brain Function and Behaviour (167a) includes a critical discussion on the role of 5-hydroxytryptamine in the central nervous system. The Herter lectures by Eccles (81) review much recent work including that from his own laboratory.

THE DISTRIBUTION OF COMPONENTS OF POSSIBLE TRANSMITTER MECHANISMS

Table I summarizes some of the available information as to the distribution of these components. The interpretation of such figures is not easy [see (110) for a discussion of some of the factors involved]. Burgen & Chipman (50a) also determined succinic dehydrogenase activity in a variety of nervous tissues, to obtain an estimate of the general metabolic activity of the tissues concerned. It is possible that a fairer idea of the significance of a given activity would be obtained from the ratio between that activity and the succinic dehydrogenase activity, as measuring roughly the content of transmitter component per unit metabolizing tissue. Certainly, if this is done, a somewhat different picture emerges.

Acetylcholine. Hebb & Silver (162) find that choline acetylase activity is limited by supply of acetyl-coenzyme A, especially with rapid rates of synthesis. Taking account of this, they have determined choline acetylase activity in the central nervous system, with results similar to those of Feldberg & Vogt (110), but obtaining much higher activity in active tissues (caudate nucleus and ventral roots can synthesize 10 to 15 mg. acetylcholine/gm. tissue/hr.) and more regular results. Their data have been used in Table I. Burgen *et al.* (49) examined the substrate specificity of the enzyme. Hebb & Smallman (163) have shown it to be concentrated in mitochondrial particles deposited at 12,000g., although the activity of the particles is low until they are treated with ether. In a sectioned nerve [Hebb & Waites, (164)] choline acetylase accumulates centrally, and falls distal to the point of section, indi-

cating that, as with cholinesterase, the main cell body is the source of enzyme. In growing rabbits and guinea-pigs (161) the choline acetylase in cerebrum rises steadily to its adult value at 30 to 100 days, but cerebellar content (much lower in the adult) reaches an early maximum and then declines. Choline acetylase can be inhibited by hemicholiniums (216) but the drug appears to interfere with choline access to the enzyme rather than with the enzyme itself (130). Bose & Gupta (28) found that feeding rats paludrine, quinine, or mepacrine lowered acetylcholine content of brain, heart and liver. The general picture of choline acetylase distribution is still compatible with Feldberg & Vogt's suggestion that cholinergic and noncholinergic neurones tend to alternate, and with the notion that the most primitive pathways (phylogenetically) are cholinergic. Cerebellar cortex, optic nerve, and dorsal roots remain outstandingly low in choline acetylase content, caudate, and ventral roots outstandingly high.

Histamine.—Histamine is known to occur in peripheral nerve and in the infundibular region, but brain and spinal cord contain little (see 92, 104, 182, 322). Feldberg & Greengard (107) have shown it to be present in cat sciatic nerve freed of its perineurium, and for a part of it to be releasable from both sites. Murray & Paton (232) have made similar observations; maximum release possible fails to interfere with conduction of motor impulses. The fact that histamine content increases rather than decreases after nerve section has prompted the latter workers to compare histamine content with Schwann cell counts; the histamine content seems to be highest in those nerves with a high ratio of Schwann cells to axoplasm, and an increase in Schwann cells is known to occur after nerve section. It seems most likely at present that the histamine is associated with Schwann cytoplasm rather than with the axone. If this is true, it may apply to other substances too; and if some of the glia can be regarded as analogous to Schwann tissue (99) distribution of certain active substances may be a function of glial arrangement as much as of specific neurones. Sympathins have already been found in gliomas, as well as cholinesterase (47).

Sympathin.—The distribution of norepinephrine and epinephrine was established by Vogt's (317) classical study, demonstrating their localization (chiefly as norepinephrine) in those regions concerned with central autonomic representation. Monoamine oxidase occurs in the brain, especially in the hypothalamus and medulla (310), but doubts are accumulating as to whether this is the main enzyme concerned in sympathin destruction.

5-Hydroxytryptamine.—Paasonen & Vogt (243) using a test object (Spisula heart) insensitive to epinephrine and pituitary hormone, have verified the conclusion of Amin *et al.* (7) that autonomic ganglia and posterior pituitary are free of 5-hydroxytryptamine. Albrecht *et al.* (3) find that 5-hydroxytryptamine of mouse brain is lower in late afternoon than in the morning. The figures for 5-hydroxytryptamine distribution in Table I are taken from Amin *et al.* (7) and Paasonen & Vogt (243). Udenfriend & Bogdanski (310) give higher values obtained by a fluorimetric method, with similar regional varia-

TABLE I

Column

Tissue	1 Acetyl- choline $\mu\text{g./gm.}$	2 Choline acetylase mg./gm. hr.	3 Q Mech.	4 Q Benz.	5 Substance P $\mu\text{g./gm.}$	6 5-hydroxy- tryptamine $\mu\text{g./gm.}$	7 HTn. decarb- oxylase $\mu\text{g./gm./hr.}$	8 Mono- amine oxidase	9 Norepi- nephrine $\mu\text{g./gm.}$	10 Hist- amine $\mu\text{g./gm.}$	11 Succinic dehydro- genase
Cortex	—	—	—	—	—	—	—	844	0.11	0	—
3 (somaesthetic)	2.8	—	150	—	—	—	—	—	0	—	—
4 (motor)	4.5	3	178	42	19	0.02	—	—	0.18	—	88
17 (visual)	2.2	1.3	107	—	7.3	0	—	—	0.04	—	—
51 (olfactory)	—	3.7	466	91	29	0.016	—	—	0.12	—	44
Hippocampus	—	—	—	—	15	0.045	—	—	—	—	—
Corpus Callosum	2.1	—	16	27	6	0	—	—	0.08	—	13
Olfactory bulb	1.3	1.2	197	—	6	0.05	—	—	—	—	—
Optic tract	—	—	86	76	—	—	—	—	—	—	—
Optic nerve	0.3	0	11	222	6	—	—	—	0.02	—	3
Caudate nucleus	2.7*	13.3	3936	360	46	0.10	0.6	—	0.06	0	134
Thalamus	3	3.1	409	161	13	0.02	—	886	—	0	90
Lateral nuc.	—	—	—	—	8.4	0?	—	—	0.08	—	—
Medial nuc.	—	—	—	—	11	0.07	—	—	0.24	—	—
Geniculates	—	—	—	—	—	—	—	—	—	—	—
lateral	—	2.6	230	—	—	—	—	—	0.07	—	—
medial	—	3.2	316	—	—	—	—	—	0.13	—	—
Hypothalamus	1.8	2.0	323	358	70	0.25	0.45	3154	1.03	1-30	—
Superior colliculi	1.65	2.3	932	159	20	0.13	—	—	0.16	—	68
Inferior colliculi	—	1.4	364	184	—	—	—	—	0.11	—	80
Red nucleus	—	—	452	—	—	—	—	—	0.26	—	—
Pons	—	—	—	—	—	—	2.6	882	0.20	—	—
Medulla	1.6	—	—	—	25	0.03	2.6	1250	—	—	—
Pyramids	0.2	—	82	94	—	0.24	0	—	0.06	—	—
Area postrema	—	—	—	—	460	—	—	—	1.04	—	—

Column

Tissue	1 Acetyl- choline μg./gm.	2 Choline acetylase mg./gm. hr.	3 Q Mech.	4 Q Benz.	5 Substance P u./gm.	6 5-hydroxy- tryptamine μg./gm.	7 HTn decarb- oxylase μg./gm./hr.	8 Mono- amine oxidase	9 Norepi- nephrine μg./gm.	10 Hist- amine μg./gm.	11 Succinic dehydro- genase
Cerebellum	0.18	0.09	1075	24	2	0.01	0	970	0.07	0	100
Inf. ped.	—	—	295	—	—	—	—	—	—	—	—
Middle ped.	—	0.33	243	50	41	0	—	—	—	—	10
Sup. ped.	—	—	333	90	—	—	—	—	—	—	—
N. grac. & Cun.	—	—	477	—	110	0.17	0.11	—	0.11	—	—
Spinal cord	1.6	—	—	—	29	0	—	—	—	0	—
Grey matter	3.0	—	611	218	68	0.08	—	—	0.16	—	—
Dorsal column	0.05	—	36	27	—	—	—	—	—	—	7
Post. roots	0.04	<0.02	—	—	40	0	—	—	0.01	—	<2
Ant. roots	15	11	149	20	6	0	—	—	0.06	—	<2
Symp. ganglion	30	—	—	—	4-9	0	45	—	6	<5	—
References	215a	162	50a	50a	7, 243	7, 243	126	310	317	182, 322	50a

* Corpus striatum.

The table has been constructed from the available evidence for dog tissues. The columns show in turn (1.) Acetylcholine content, in μg./gm. fresh tissue; (2.) Choline acetylase activity, in mg./acetylcholine synthesized/gm. wet wt./hour; (3.) Rate of hydrolysis of methacholine, in μl. CO₂ evolved/gm. wet wt./10 min., as an estimate of true cholinesterase activity; (4.) Rate of hydrolysis of benzoylcholine, in μl. CO₂ evolved/gm. wet wt./10 min., as an estimate of pseudo-cholinesterase activity; (5.) Substance P content, in units/gm. wet wt.; (6.) 5-hydroxytryptamine content, in μg./gm. wet wt.; (7.) 5-hydroxytryptophan decarboxylase activity, in μg. 5-hydroxytryptamine formed/gm. tissue wet wt./hr.; (8.) Monoamine oxidase activity, in μg. 5-hydroxytryptamine destroyed/gm. homogenized tissue/hr.; (9.) Norepinephrine content, in μg./gm. wet wt.; (10.) Histamine content, in μg./gm. wet wt.; (11.) Succinic dehydrogenase activity as a percentage of activity of cerebellar hemispheres, which had an absolute activity of 167 μg. succinic acid dehydrogenated/gm. wet wt./hr.

In columns 1 to 6 and 9, the four highest values in each column are indicated by italicized figures.

tions. Gaddum & Giarman (126) have studied the distribution of 5-hydroxytryptaphan decarboxylase. It is widespread in the alimentary tract; the kidney is particularly rich (45 to 200 μ g. 5-hydroxytryptamine formed/gm./hr.), but plasma is free of it. Sympathetic ganglia are very rich, and the cerebral peduncles, pons, medulla, hypothalamus, and caudate nucleus contain it. The substrate concentration/action curve is bell-shaped for the enzyme from nervous tissue. Curious anomalies exist, area postrema is rich in 5-hydroxytryptamine, low in enzyme; autonomic ganglia are free of 5-hydroxytryptamine but rich in enzyme; the caudate and hypothalamus contain both. Monoamine oxidase, which can destroy 5-hydroxytryptamine, is especially concentrated in hypothalamus, and to a less extent the medulla (310). In general the distribution of the 5-hydroxytryptamine system is not dissimilar from that of sympathin.

Substance P.—Paasonen & Vogt (243) agree with earlier work that hypothalamus and caudate contain large amounts. Area postrema may have considerably higher values than the ala cinerea, and the ala cinerea than the trigonum hypoglossi. P has also been found in fish intestine and brain (von Euler & Ostlund, 93); and Eliasson *et al.* (88) have failed to substantiate the suggestion that P from the intestine differs from that in brain. It is known to be present in retina (79). Substance P synthesis and metabolism have hardly been studied; although presumably normal pathways for peptide synthesis and breakdown are concerned. Umrath has speculated that P is the carrier for acetylcholine (in bound form) in cholinergic neurones, and for the sensory transmitter in dorsal roots (312). It is curious that the distribution of substance P is not unlike that of the 5-hydroxytryptamine system and of sympathin except for its concentration in dorsal roots and their central pathway. The area postrema may contain more substance P than does any other tissue.

The inhibitor substance of Florey (I).—This material (89, 112, 113) obtained by extraction of brain with boiling water, followed by dialysis, concentration and further purification, is assayed by its depressant action on the discharge of the crayfish stretch-receptor. It is distinguished from acetylcholine, substance P, histamine, sympathin, 5-hydroxytryptamine and ATP, and is identified as γ -amino- β -hydroxy-butyric acid. It is remarkable that Hayashi & Nagai (160), in a study of compounds of this type for anti-convulsant activity when applied to the cortex, selected this particular compound as being a possible central inhibitor. Hanawara (152) has studied retinal metabolism, and the effect of various amino acids. Florey & McLennan (112, 113) found that Florey's inhibitory substance (I) is not destroyed in blood, and that it does not occur in sympathetic ganglia, sciatic nerve, liver, spleen, or heart. It accumulates in fluid placed in a cup over the cortex of a cat, with dura opened. Knowledge about γ -aminobutyric acid and similar compounds is rapidly accumulating; a valuable critical survey is that of Elliott (88a).

Other substances.—Crossland & Mitchell (73) have obtained, from trichloroacetic acid extracts of cerebellum at room temperature, a material

which when injected into the carotid artery produces cerebellar activation. It is very stable at alkaline pH but can be destroyed by boiling with acid. Extracts of cerebellum also destroy it, but not if they are previously boiled. The active material is found in optic nerve and dorsal roots.

Ambache (5) has further characterized Irin, a substance extracted from iris and believed to mediate the prolonged pupillary constriction resulting from handling the trigeminal nerve. It is distinct from acetylcholine, histamine, 5-hydroxytryptamine, bradykinin and substance P. Its action is long-lasting, up to several hours. From partition experiments it appears to be an acid.

Vogt (318) has shown that *Darmstoff*, a smooth muscle stimulant, extractable from intestine, has the properties and chemical properties of a phosphatidic acetal. In an interesting paper he reviews the lipoid-soluble acids of natural occurrence, and suggests that such compounds might act as mobile cation-exchangers in cell-membranes. Sphingosine (114) (a base in sphingomyelin) has been found to have a significant, but irreversible, spasmolytic action on isolated intestine. It may be relevant that White & Sanson (325) find that injections of fatty acid anions can produce EEG spindling and the appearance of sleep.

Lissak & Endroczi (207), from watery extracts of nervous tissue, obtained a substance spasmolytic on the small intestine, and depressing cortical excitability and spinal reflexes; it protects mice against strychnine and eserine convulsions. It is dialyzable, but is broken down in aqueous solution. Kuriaki & Haraguchi (196) describe a lipoid soluble vasodilator in brain. Regional difference in content of glycerylphosphoryl choline (320) and of porphyrins (295) may also become important.

Comment.—Despite the undoubted importance attaching to any naturally occurring substance, none of the substances mentioned, save acetylcholine, commend themselves as transmitters for specific neural responses, because of their relatively prolonged action or their evident chemical stability. Central synaptic events are commonly both rapidly repetitive (firing rates up to 1000/sec. are described) and relatively transient in their course; only after prolonged repetitive stimulation do events with a time course of minutes occur, and these may well be due to ionic shifts associated with propagated activity in somata, dendrites, and axones rather than to transmitter accumulation. Transience of synaptic action is, in any case, necessary if inextricable blurring of successive signals is not to occur; and the results of prolonged transmitter action at the neuromuscular junction (54, 303, 304, 305) indicate that synaptic function might be radically disorganized if transmitters had other than a transient action. This is not a decisive argument, since the required transience of action might be achieved by release at point sources and diffusion away from them. But the weight of the argument is proportional to the significance attached, for instance, to cholinesterase. The reviewer suspects that the substances identified may bear the same sort of relation to undiscovered transmitters as choline does to acetylcholine. The appearance of pharmacologically active anions is of particular interest.

As mediators for diffuse responses, particularly of the reticular formation,

sympathin, 5-hydroxytryptamine, substance P, offer themselves, in addition to any role acetylcholine may play. P is usually discussed in terms of transmission by the first sensory neurone; but apart from its presence there, the fact that, like 5-hydroxytryptamine and sympathin, it is concentrated in area postrema and hypothalamus, and (relatively) in medial rather than lateral thalamus, gives it almost an equally good (or bad) claim with these for a reticular function. Vogt's valuable critical discussion (317) of the possible function of cerebral sympathin is still relevant to the other possible transmitters.

RELEASE OF TRANSMITTERS

Transmitter substances.—Angelucci (8) has used the oxygen-perfused, Ringer-bathed, frog spinal cord preparation, from which flexor reflexes, inhibited by contralateral excitation, can be obtained. On stimulation, increases of acetylcholine output (in the presence of eserine), and of substances resembling 5-hydroxytryptamine and substance P, were obtained in small quantities. Unexpectedly, reserpine (which is known to mobilize 5-hydroxytryptamine from platelets, intestine, and brain) lowered the output of 5-hydroxytryptamine-like substance.

Acetylcholine output has been studied principally on peripheral structures. Emmelin & MacIntosh (90) have verified that acetylcholine output in ganglia or muscles perfused with blood is comparable with output from Locke-perfused tissues, and have discussed critically the nature of events in chemical transmission. Kostial & Vouk (193) find that for an excitation rate of 2/sec., acetylcholine output from a cat's perfused superior cervical ganglion is little reduced by cooling unless to 20°C. or lower. They suggest that Brown's (44) earlier observation that output fell to one-tenth between 39°C. and 20°C. was related to his more rapid stimulus rate (10/sec), with which the rate of acetylcholine resynthesis might have become limiting and caused a greater temperature sensitivity. Studies on miniature endplate potentials have continued (30, 56a, 124a, 204). The externally recorded potential difference may exceed that recorded across the endplate membrane because of a concentration of current flow in the synaptic gap (56a). Such potentials have not been seen in autonomic ganglia (81) so that it is not established whether acetylcholine release is also quantal at interneuronal synapses, although it is hard to imagine that it is not.

Paton (252, 253) has shown that the output of acetylcholine by guinea pig ileum is considerably increased by coaxial stimulation, which elicits brief twitches, having the pharmacological properties of a response mediated by postganglionic cholinergic neurones (250). No other gut stimulant was released in significant amounts. Although it is known that histamine, 5-hydroxytryptamine and substance P are present in intestine, as in the brain stem, the nervous transmission of stimulant effects by the nerve network of the intestine appears to be wholly cholinergic.

Gillespie & Brown (135), in a study of sympathin output from spleen and

gut, made the interesting observation that treatment with dibenamine increased the amount of norepinephrine recovered; they interpret this to mean that the receptors destroy as well as receive the transmitter. This situation contrasts with cholinergic synapses, at which receptors can be blocked without interfering with the amount of transmitter collected.

Accommodation to acetylcholine.—Conventional theories of drug action usually assume that the activity of a drug bears some relation to the occupation of receptor sites by the drug concerned, so that as the proportion of sites occupied reaches some equilibrium value the effect of the drug achieves a stable sustained peak. In fact, however, a sustained action by a drug is almost impossible to achieve. Drugs such as histamine, hydroxytryptamine, and Substance P, when applied to the small intestine, elicit a waning contraction and if a large dose is applied and subsequently washed out a desensitization to the drug is obtained which is often specific if the dose is carefully chosen. This type of phenomenon often comes under the heading of "tachyphylaxis" and forms the basis of the Barsoum-Gaddum desensitization method for identifying substances in the absence of a specific inhibitor. A similar accommodation to the stimulant drug at the motor endplate occurs with acetylcholine and drugs like it. It has been repeatedly recorded that the effect of acetylcholine or decamethonium at an endplate will produce during a particular administration a waning depolarization, and a smaller effect on subsequent administrations, even when apparent complete recovery of membrane potential has taken place between successive doses (54, 78, 101). A striking example of this is the observation by Thesleff (303, 304, 305) that in a frog sartorius exposed to acetylcholine the initial endplate depolarization (measured with internal electrodes) may with lapse of time entirely disappear. Krivoy & Wills (194) record a curious accommodation in isolated ganglia whereby acetylcholine produces a sustained depolarization but transmission returns (the exact converse of Thesleff's observation). Pascoe (246), however, found acetylcholine action to lessen with successive applications. Thesleff believes that acetylcholine can produce a competitive block comparable to that produced by d-tubocurarine. The muscle will certainly have lost appreciable quantities of potassium (189, 251) and other ion movements have probably occurred. But since the membrane conductance of the muscle in this state is still within normal limits, the defect must be in a failure by acetylcholine to produce permeability changes, rather than in a change of possible equilibrium potentials. A similar phenomenon has been found on the frog rectus abdominis muscle when its response to acetylcholine is studied, not by allowing it to contract against an isotonic lever, but by stretching the muscle to a constant length and finding the tension developed (254). Under these conditions the "active state" induced by the drug wanes with time, sometimes quite rapidly, and the muscle becomes eventually completely resistant to acetylcholine. Sensitivity is only slowly recovered after washing out. Since acetylcholine should be rapidly destroyed at the nerve-endings and could hardly be expected to persist, as, say, d-tubocurarine

can, it seems more likely that acetylcholine induced some "refractoriness" of the receptors rather than that it exerts an ordinary curare-like action. A very interesting related clinical development is described by Churchill-Davidson & Richardson (59). After noting that the condition of a myasthenic patient deteriorated with activity, and that the effect of anticholinesterases was ultimately to make her worse after a temporary improvement, they concluded that the myasthenic motor endplate changed its response to acetylcholine, so that acetylcholine (or its products) came to curarise the endplate. Zaimis (335) on the basis of experiments with depolarizing drugs on the muscles of a variety of animals, put forward a similar proposal and Grob *et al.* (147) have made kindred observations. Churchill-Davidson and Richardson accordingly "rested" the endplates by curarising the patient for a period of eight days. The curare requirement was soon within normal limits. At the end of this period as the curare wore off the patient improved remarkably, and neostigmine had a dramatic effect, the patient becoming stronger than for a long time.

Although accommodation of central nervous structures to the action of stimulant drugs has been little studied, it may be of considerable importance in interpreting some of the results obtained. Any drug action, in which more than brief effects are obtained and in which the response changes, for instance, from stimulant to inhibitory, may involve such a process.

MECHANISMS INVOLVED IN THE GENESIS OF BIOELECTRIC POTENTIALS

Peripheral nerve.—Hodgkin & Keynes (168, 169) showed in 1955 that after prolonged stimulation of a squid axone, the process of sodium extrusion and potassium recovery were coupled, and were "active" in the sense of proceeding against electrochemical gradients and being paralyzed by metabolic inhibitors. As a result of their work on dinitrophenol-poisoned fibres, for which potassium flux ratios of 0.1 were obtained, they concluded that K^+ ions undergoing passive diffusion interacted as though they had to traverse, in single file, channels through the membrane accommodating about three ions. Subsequently (170) they have developed a refined method for injection inside axones; KCl so administered had little effect, but NaCl led to a reduction of "overshoot" and a small rise in resting potential attributable to accelerated Na^+ extrusion. They were able to show that Na efflux is proportional to intracellular sodium, Na_i , although the constant of proportionality falls with time: the onset of accelerated efflux after NaCl injection was immediate. $MgCl_2$ and d-tubocurarine were inactive, but $CaCl_2$ abolished conduction and rendered the axoplasm fluid and opaque. The "toxicity" of intraaxonal calcium agrees with Keynes & Lewis' (185) conclusion that the concentration of Ca in squid axoplasm cannot be above 0.1–0.3 molar. The latter authors, with Gilbert & Fenn (134) suggest that calcium is actively extruded from the fibre. Frankenhaeuser (119) has further shown that reduction of extracellular calcium, Ca_o , to zero will paralyze conduction in frog myelinated nerve, although 0.01 mM is sufficient to maintain excitability.

Frankenhaeuser & Hodgkin (121), have shown that the external calcium profoundly influences Na and K exchanges; a fivefold reduction of Ca_o is equivalent (in its action in facilitating increase in sodium conductance, inactivation of the sodium-carrying system, and rise in K conductance) to a membrane depolarization of 10 to 15 mV.

Afterpotentials in peripheral nerve.—Brown & Holmes (45), and Ritchie & Straub (266, 267) have made a very interesting analysis of the afterpotentials following repetitive stimulation of mammalian C fibres (cervical sympathetic and hypogastric nerves). After such stimulation, the recorded spike and the size and duration of the afternegativity are increased and a large afterpositivity appears, as Gasser & Grundfest had earlier shown. The change in spike height is due, not to a change in absolute height, but to the spike "taking-off" from the afterpositivity, and to an increasing contribution to the recorded spike by the enhanced afternegativity, particularly if dispersion is increased by long conduction distances. The afternegativity appears only as the hyperpolarization develops. Brown & Holmes find that the phenomenon is prominent for all fibres conducting less than 1.1 m/sec, and believe it to be a property of nonmyelinated nerves. Analyzing the hyperpolarization, Ritchie & Straub found it to be delayed in high external potassium; reduced by low external potassium, by dinitrophenol and other metabolic inhibitors, by ouabain, and by substituting lithium for sodium; prolonged in sucrose or choline Na-deficient media; and independent of external chloride concentration. After metabolic inhibitors and lithium, post-tetanic depolarization replaces the normal hyperpolarization. Holmes (172) studying the post-tetanic enhancement of the action potential, obtained, on cat hypogastric nerve, similar results in some respects, but with a lower dose of DNP found it produced only a delay in time course of the enhancement. Ritchie & Straub propose that during excitation, a relatively large quantity of sodium enters the fibres, and potassium leaves (some of which diffuses away). During recovery sodium extrusion coupled to potassium influx lowers extracellular potassium, K_o , immediately outside the fibres faster than it can be replaced by diffusion from surrounding fluid; K_i/K_o thus temporarily rises above normal levels, leading to the hyperpolarization. The theory explains the phenomena described, and the fact that the hyperpolarization approaches but does not exceed the membrane potential recorded in K-free solution. The theory assumes a diffusion barrier round the nerve, and predicts that the hyperpolarization will develop in proportion to the surface-volume ratio of the fibre. Holmes (172), however, interprets his experiments in terms of a prolonged K conductance following the passage of a train of impulses.

Frankenhaeuser & Hodgkin (120) in a study of the effects of nerve impulses in squid axon also invoke a diffusion barrier. They noticed that the afterpositivity of the spike in a train dwindles as the train continues; this effect is larger with low K_o and is reduced by high K_o and by rise of temperature (which reduces the K output per shock). A diffusion barrier, spaced

about 300 Å from the membrane (it need contribute only slightly to the total membrane resistance), behind which K^+ may accumulate, explains the results, together with certain voltage-clamp observations; and Hodgkin and Huxley's theory of the nerve impulse can be extended to include the negative after-potential. Frankenhaeuser and Hodgkin found, in electron-microphotographs obtained by B. B. Geren & F. O. Schmitt, a barrier of the position and nature postulated.

Shaw *et al.* (283), having failed to obtain the expected correlation between K_i and Na_i and the resting or action potential respectively, criticize the current theory of membrane potential. Katz (183) criticizes Easton's (80) suggestion that the effect of nerve stimulation on the muscle action potential recorded at the endplate is due to current from adjacent fibres, and shows that if the membrane potential is raised so that the impaled fibre will not conduct an impulse from the endplate, the endplate potential recorded is of normal shape, despite activity in adjacent fibres.

Neurones of the central nervous system.—Eccles (81) has reviewed the recent experiments in his laboratory which have shed so much light on neuronal physiology. In the resting state, the neurone has a membrane potential of 70 mV, about 20 mV less than the K^+ equilibrium potential, implying normal resting K^+ intrusion. When activated antidromically, the full spike response is slowed in both rising and falling phases by injection of Na^+ ion (which raises Na_i and lowers K_i), consistent with the Hodgkin-Huxley theory for propagated action in nerve. During synaptic activation the equilibrium potential approaches zero; the depolarizing excitatory postsynaptic potential (e.p.s.p.) is largely proportional to the membrane potential; and the injection of various ions had no specific effect; all these results indicate a "short-circuit" resulting from synaptic action. During inhibitory responses the hyperpolarizing inhibitory postsynaptic potential (i.p.s.p.) was found to have an equilibrium potential about -80 mV, and responses to injected ions were compatible with a specific increase of permeability to small ions (K and Cl). The positive afterpotential following antidromic excitation had an equilibrium potential of -90 mV and other properties corresponding to a persisting K permeability. Four specific permeability changes are thus envisaged, such that the membrane potential is dominated by the movement down electrochemical gradients of sodium (rising phase of spike), potassium (falling phase and afterpositivity), all small ions (inhibitory postsynaptic potential) or all ions (excitatory postsynaptic potential).

Fatt's (102) analysis of potentials round a single activated motoneurone proposes a reidentification of the components of potentials recorded. The response elicited had (after a small initial positivity) two phases: a negative deflection culminating in a plateau, from which arose a further negative spike. The first plateau was identified as soma response because (a) it was maximal nearest where penetration of the neurone occurred, (b) it resembled soma intracellular records, (c) it was a large potential requiring a large source, (d) it did not propagate. Given this conclusion, the spike which follows the

plateau and may fail to appear in blocked neurone, could hardly be other than dendritic. This was confirmed by finding evidence of propagation of the spike at about 1 m./sec. The threshold for soma activation was about 10 mV, and its height 30 mV; dendritic response was elicited at 30 mV, giving a spike of 80 mV. Fatt concludes that the NM (nonmedulated part of axone) response of Brock *et al.* (36) (which Eccles has now rechristened IS for initial segment) is in fact somatic; that the SD (soma-dendrite) response is dendritic; and that it is the soma-dendrite junction rather than the axon-hillock that is a point of easy block.

This conclusion was supplemented by orthodromic studies (103); potential steps can be identified (particularly clearly by electrically differentiating the record) in synaptic and intracellular spikes, corresponding to the successive activation of the soma and of the dendrites. Fatt agrees with Brock *et al.* (36) that a partly blocked antidromic spike appears to sum with the synaptic potential (although a full antidromic spike abolishes it), but suggests that this apparent summation might involve the appearance of a local response. The distribution of extracellular synaptic potentials was diffuser than that for antidromic excitation, suggesting that dendrites are also involved in synaptic action. It was also concluded that the dendrites had a longer time constant (c.4msec.) than the soma (2msec.) and that the length of the synaptic potential might be due to this rather than to prolongation of transmitter action as proposed by Coombs, Curtis & Eccles (68).

Fuortes, Frank & Becker (124) have also studied the origin of motoneurone spikes with intracellular electrodes (but not by mapping the external field). They find that an inflection in the rising phase of the spike is present when the motoneurone is excited by a short pulse as well as antidromically. The partially blocked spike can still make the axone discharge (as tested by axone refractoriness). They still take the view that the more readily excitable area of the neurone (A area) is in the region of the axone hillock, although it might be "small spots scattered over soma and dendrites." Crain's (71) work on cultured spinal ganglion cells may be relevant; these have no dendrites but a single repeatedly branching "neurite." The cells have resting potentials up to 65 mV, spikes up to 95 mV, and are not spontaneously active. Excited by electrodes thrust into the culture, the response (according to stimulus strength or frequency) may be a modest "local response," or a larger "prepotential" from which a spike may arise, having a humped falling phase, and lasting up to 4 msec., followed by a positivity.

Dendrites.—The difference in time constants reported by Fatt recalls Burns' (52) suggestion, backed by a working model, that repetitive discharges of nerve cells might originate in cells in which, after activation, one part (soma) repolarized more quickly than the rest (dendrites), allowing repeated self-activation. Clare & Bishop (60) and Landau (197) showed that cortical dendrites conducted only centrifugally; had no refractory period; exhibited a cycle of enhanced excitability during the surface negative re-

sponse, reduced excitability during the positivity, and increased excitability again thereafter; and could sum up depolarizations. After strychnine applied in high concentration to the cortex (62, 197), dendrite activity is depressed, and surface-positive responses alone may be recorded to thalamic or cortical stimulation. Grundfest & Purpura (149) find that d-tubocurarine injected intravenously will abolish dendritic potentials both to synaptic excitation and to cortical excitation; and conclude that dendrites are excitable only by synapses and not directly by electrical currents. Although some of these conclusions seem incompatible, the concept of the dendrites as structures akin to motor endplates, imparting the product of a graded summation of chemical or electrical stimuli or both to the soma, from which renewed excitation may return, is of great interest.

PHARMACOLOGICAL EVIDENCE FOR CHEMICAL TRANSMISSION: CHOLINERGIC TRANSMISSION

Renshaw cell activation.—Eccles and his colleagues (82) have shown that these cells fire repetitively for several seconds after intraarterial acetylcholine (10 to 100 μ g) or nicotine (0.2–2 μ g); eserine potentiates the former, not the latter. Dihydro- β -erythroidine 0.4 mg./kg. exerts a 10-fold antagonism to both stimulants, and reduces the Renshaw cell response to an antidromic volley. Suxamethonium, decamethonium, methacholine, arecoline, neostigmine, octamethylpyrophosphoramide (OMPA), gallamine, tubocurarine, hexamethonium and lobeline were inactive. Strychnine did not affect Renshaw cell discharge, but pentobarbitone could reduce it. This study of unit activity was supplemented by reflex studies (74) revealing impressively similar drug effects. Contrary to some earlier work (106, 174), both flexor and extensor, and both monosynaptic and polysynaptic reflexes were depressed by acetylcholine: with intact dorsal roots augmented reflex discharges were sometimes observed, abolished by dorsal root section. They suggest that failure to section dorsal roots may allow a cholinergic activation of peripheral structures (nerve endings or muscle spindles). They failed to discover in the dorsal horn or intermediate nucleus any cells other than Renshaw cells which could be activated by acetylcholine, and conclude that these cells alone mediate the effects described.

Caudate nucleus.—Intracarotid injections of diisopropylfluorophosphate produce compulsive circling in rabbits, which is associated with an asymmetrical inhibition of cholinesterase in caudate nucleus and frontal cortex (but not of other brain areas) (9). Direct injection of diisopropylfluorophosphate into the caudate, or local stimulation elicit contraversive turning in rabbits, as do methacholine, and metrazol (324). To produce localized specific effects the cholinesterase of the caudate must be reduced to 21 to 40 per cent normal; atropine abolishes the effect (323). Ablation of the caudate is known to cause ipsiversion. Forman & Ward (115) find that stereotyped contralateral movements produced by caudate excitation are unchanged by removal of the cortex. The evidence suggests that cholinergic

tive neurones in the caudate inhibit the upper brain-stem facilitating reticular formation.

Reticular formation.—Much of the recent work was reviewed by Dell & Bonvallet (75), Bovet & Longo (29), and Brücke (46) at the Brussels Congress. Grutta & Desmedt (76), showed, on *encephale isolé*, *cerveau isolé*, or prebulbar sectioned preparations (cat) that a number of anticholinesterases injected into the cerebral circulation could produce the cortical desynchronization, ocular signs, and reduction of auditory-evoked cortical response, characteristic of arousal. The drugs used had an activity proportional to their potency against pseudo cholinesterase, although their neuromuscular action varied according to activity on true cholinesterase. Possibly glial cells, known to be rich in pseudo cholinesterase are involved. This work seems to suggest either that pseudo cholinesterase in cells not directly involved in a synapse plays a prominent synaptic role in the central nervous system or that the inhibitors are interfering with the hydrolysis of some other ester. Longo & Silvestrini (213) found that acetylcholine by carotid injection in unanaesthetized rabbits produced arousal; eserine had little action; acetylcholine was ineffective on *cerveau isolé*. It does not seem quite clear how far direct cortical action by acetylcholine or by cholinesterase-inhibitors may cause these effects although Desmedt & Schlag (77) observed increased activity in some tegmental neurones after eserine, after section rostral to the mammillary bodies. In addition, nonspecific effects must be considered. Eccles' caution (74) about afferent stimulation was mentioned above. Nakao, Ballim and Gellhorn (236) found that the increase in cortical fast waves produced by acetylcholine was abolished by section of sino-aortic nerves (suggesting that during acetylcholine action, impulses from the baroreceptors inhibitory to the hypothalamus were withdrawn). Schlag's observation (277) that the depression by acetylcholine of the pyramidal discharge to cortical stimulation (especially the I waves) could be imitated by histamine, nicotinic acid and amyl nitrite, also emphasizes the possible role of vasodilatation.

Turning to acetylcholine antagonists, Longo (212) found that atropine and hyoscine could produce cortical slow waves, and block electrical arousal to hypothalamic or fast thalamic stimulation, although the neurovegetative responses to hypothalamic stimulation, the cortical response to light, the recruiting response from the thalamus, and seizure activity to hippocampal stimulation, were unchanged. Atropine can also remove the slow cortical waves seen after electroconvulsion therapy (311), and abolishes the tremor-inducing action of Tremorine (97).

The correlation of electroencephalograph studies and behavior, however, has showed that "electrical" and actual arousal may not correspond. Bradley & Hance (33) find that amphetamine and lysergic acid diethylamide (LSD), given to animals with implanted electrodes, will indeed produce both fast waves and alert restless behavior; and that chlorpromazine can produce slow waves and "sedation," and antagonize the former drugs. But

eserine, on the other hand, will produce fast waves without behavioral alertness; and if eserine is given with chlorpromazine, fast waves may coexist with a well-developed chlorpromazine sedation. Likewise atropine produces slow waves, but no sedation, and can abolish electrical arousal, although behavioral arousal takes place. When members of the two groups of drugs, i.e. LSD, amphetamine, or chlorpromazine on the one hand, eserine, or atropine on the other, are combined, it seems that the former determine the physiological state, the latter the electrical record.

Medulla.—Diisopropylfluorophosphate injections into the medulla cause turning movements (324): and tetraethyl pyrophosphate (TEPP) injected into the fourth ventricle causes a considerable increase in the hyperpnoea to stimulation of Hering's nerve (225).

ADRENERGIC TRANSMISSION

Spinal cord.—Previously Schweitzer & Wright (281) had found epinephrine to depress the knee jerk. Burn, Bülbring & Skoglund (51) observed variable effects, and obtained evidence that the presence or absence of adrenaline modified cord responses. Bernhard & Skoglund (20) showed that both epinephrine and norepinephrine increased the monosynaptic reflex (and the P wave of the cord potential), but reduced flexor mono- and multi-synaptic reflexes. Wilson (328) finds that epinephrine increases the monosynaptic reflex in low doses, but with high doses depresses it. Eccles and his colleagues (74), however, could record no effects with epinephrine save late ones, possibly due to vascular changes. Angelucci (8) failed, even with epinephrine 10^{-4} , to affect reflexes of frog spinal cord.

Reticular system.—Dell & Bonvallet (75) and Bovet & Longo (29) review much of the work implicating adrenoceptive structures in reticular arousal. Slocombe, Hoagland & Toazian (290) find that the anaesthetic used is critical; in the animal under thiopentone, epinephrine reduces frequency and amplitude of cortical activity, but not in the etherized animal. Nakao *et al.* (236) find that, in cats under light thiopentone anaesthesia, epinephrine and norepinephrine increase the slow potentials in the cortex, producing a picture like full barbiturate anaesthesia; this change is abolished by sino-aortic denervation, and they think that excitant action on the reticulum is seen only with big doses. Rothballer (270) records both cortical "activation" and "deactivation" by epinephrine in unanaesthetized cats; after thermocoagulation of the pontomesencephalic reticulum epinephrine was effective, but carrying the lesion up to mesodiencephalic border abolished epinephrine effects. Longo & Silvestrini (213) found that epinephrine in rabbit had little activating action, and might sedate. Milösević (226) finds that epinephrine intensifies both the anaesthesia produced by thialbarbitone and other anaesthetics, and also the convulsant action of strychnine, nicotine, and cocaine. Among nonspecific effects, it is worth noting Schmitt's (278) observation that although with high blood pressures small doses of epinephrine and norepinephrine reduce brain volume, after lowering the blood pressure the

same doses produce a large rise in cerebral volume; the volume changes are often more prolonged than the blood-pressure effects. Abrahams & Pickford (1) find that epinephrine in low dosage can prevent the antidiuretic effect of acetylcholine, but high doses do not do this. They discuss the possibility of a neurone inhibiting the cholinceptive supraoptic neurones, stimulated by low doses and paralyzed by high doses of epinephrine.

It seems to the reviewer, taking also into account Marrazzi & Hart's (220) demonstration that epinephrine paralyzes transcallosal transmission and the action of epinephrine on ganglia (223) that excitation (as opposed to facilitation) of nerve-cells by epinephrine is still to be demonstrated. It may be noted that although amphetamine is often regarded as sympathomimetic, this is probably incorrect, and experiments with it cannot necessarily be used as evidence of adrenoceptive processes; amphetamine appears to have closer connections to 5-hydroxytryptamine (314). A modulating action by epinephrine is more readily accepted as a possibility. Loewenstein (211), on the basis of the effect of sympathetic stimulation and of epinephrine on sensory discharges from frog skin, goes so far as to coin the term "modulapse" for the situation where the function of one neurone is modulated by another, in the absence of normal transmission. But since applied currents (anode outside) have the same action, and epinephrine can reduce the standing potential (outside negative) across frog skin, the action of epinephrine here may arise from reduction of the skin polarization rather than from any specific neuronal action.

TRYPTAMINERGIC TRANSMISSION

Gaddum (125) has reviewed the distribution and action of 5-hydroxytryptamine (HT). Gaddum & Vogt (127) have confirmed Feldberg and Sherwood's observation that 5-hydroxytryptamine intraventricularly produces a lethargic state, although they found it persisted several hours. The other actions of 5-hydroxytryptamine described earlier (potentiation of barbiturate anaesthesia, antagonism to strychnine, reduction of flexor reflexes, reduction of transcallosal transmission) are also largely depressant, although on autonomic ganglia a stimulant action can be demonstrated (306, 307), and 5-hydroxytryptamine can excite sensory endings (10). Release of an 5-hydroxytryptamine-like substance from the frog spinal cord, increased by reflex excitation, was reported by Angelucci (8). Paasonen & Vogt (243) find that brain 5-hydroxytryptamine is not changed by administration of ephedrine, ether, insulin, or β -tetranaphthylamine, but amphetamine reduces it about 50 per cent. Fastier *et al.* suggest (100) that hypnosis-prolongation by 5-hydroxytryptamine may be secondary to its hypothermic effects. Bonnycastle *et al.* (27) find that a number of anticonvulsants raise 5-hydroxytryptamine in brain (but not in gut or spleen); but raising 5-hydroxytryptamine by giving iproniazid or 5-hydroxytryptophan did not protect against leptazol convulsions.

The action of 5-hydroxytryptamine has been inculcated in the effects

produced by other drugs, especially reserpine (for a general review see ref. 16) and lysergic acid diethylamide (LSD), which deserve separate discussion.

Reserpine.—The interpretation of reserpine action only in terms of 5-hydroxytryptamine mobilization must be modified since Muscholl & Vogt's (234) discovery that sympathin as well as 5-hydroxytryptamine is released in the brain. Falls in plasma 5-hydroxytryptamine (146) and plasma sympathins (181) after reserpine are also reported. In an animal treated with iproniazid, reserpine does not reduce brain 5-hydroxytryptamine, and its action becomes more like that of lysergic acid diethylamide (58, 286). Reserpine is known to elicit the alerting reaction (265), to facilitate convulsants such as camphor, caffeine, nikethamide, leptazol, and strychnine or electric shock (22, 57) (although anticonvulsant action can be detected, 57), to antagonize anticonvulsants (57), to antagonize morphine analgesia, to produce emesis in pigeons, and to increase the monosynaptic reflex (280). On the other hand, it can depress the "search and flight" reaction of the rabbit to hypothalamic stimulation (238), reduce sham rage in the cat (280), depress the reflexes of frog spinal cord (8), modify conditioned avoidance behavior in monkeys (292), prolong barbiturate anaesthesia (an action prevented by the bromo derivative of lysergic acid diethylamide (BOL) and lysergic acid diethylamide) (274) and, if given subcutaneously, synergize with 5-hydroxytryptamine given intraventricularly (127). Reserpine has a modest spasmolytic action reversed by components of the tricarboxylic acid cycle (136) but a negligible anti-cholinesterase action (284).

Gangloff & Monnier (129) find 5-hydroxytryptamine more like reserpine than chlorpromazine in its central effects. But (23) 5-hydroxytryptamine lacks both the ability of reserpine to synergize with convulsants, and also the ability to modify this action of reserpine: this argues against reserpine action being due either to a temporary flooding of the central nervous system with 5-hydroxytryptamine, or to a subsequent deprivation of the area of 5-hydroxytryptamine resulting in increased excitability. Brodie *et al.* (38) made the interesting suggestion that the inability of the brain to bind 5-hydroxytryptamine after reserpine, combined with continued 5-hydroxytryptamine synthesis, leads to a persistent low level of 5-hydroxytryptamine and that this, rather than reduction of stored 5-hydroxytryptamine produces reserpine's effects. It is not clear, however, why, after an initial flooding, the level of free 5-hydroxytryptamine should be any higher than in the normal animal unless the rate of 5-hydroxytryptamine formation varies inversely with level of bound 5-hydroxytryptamine. No evidence for such a control of 5-hydroxytryptamine synthesis yet exists.

Reserpine action may become interpretable in terms of a conjoint 5-hydroxytryptamine and sympathin release, leading to an initial flooding of the central autonomic regions with these hormones, followed by a local lack, and a peripheral sympathetic inactivity from lack of sympathin in post-ganglionic neurones. But the cortex may also be involved, since reserpine's depressant action on conditioned avoidance by monkeys is reduced by cor-

tical ablation, especially of the temporal lobe (292); and Schneider *et al.* (280) concluded from observations on thalamic cats that the cortex was necessary for some of reserpine's actions, perhaps because the drug intensified cortical inhibition of the diencephalon.

Lysergic acid diethylamide (LSD).—The discovery that lysergic acid diethylamide was a powerful antagonist to 5-hydroxytryptamine on blood vessels, bronchi, rat uterus, and guinea pig ileum (D receptors only), prompted the notion that its hallucigenicity was caused by such an antagonism centrally (125). This view now seems less likely for the following reasons: (a) hallucinations are produced by other indolic compounds of feeble anti-5-hydroxytryptamine action (harmine, hashish, adrenochrome, bufotenin) and by some quite unrelated substances (e.g. nalorphine) (167); (b) other effective anti-5-hydroxytryptamine substances (such as the bromoderivative, BOL) fail to reproduce the characteristic LSD actions (127, 273); c) although lysergic acid diethylamide can temporarily reverse the lethargy induced by intraventricular 5-hydroxytryptamine, other drugs such as morphine and methadone can do it equally well, and 5-hydroxytryptamine cannot reduce lysergic acid diethylamide action (127).

It might be suggested, on the other hand, that the action of lysergic acid diethylamide on the brain is 5-hydroxytryptamine-like, since in appropriate dose it can stimulate smooth muscle (125) and its hallucinogenic action is abolished by BOL (137); Marrazzi & Hart (220) found that lysergic acid diethylamide and 5-hydroxytryptamine both depressed transcallosal transmission, and Slocombe *et al.* (290) found that lysergic acid diethylamide and 5-hydroxytryptamine, had similar actions on cortical activity in the rat under pentothal. But the stimulant actions of lysergic acid diethylamide, causing electrical and behavioral arousal (33), brief sham rage when given intraventricularly (127), characteristic disturbances in waltzing mice, spiders and fighting fish, and a pyrogenic-type reaction of diencephalic origin (125, 240), cannot be matched by 5-hydroxytryptamine. On this view, too, it is hard to explain the actions shared by LSD and BOL, in antagonizing the potentiation of hexobarbitone, anaesthesia by 5-hydroxytryptamine or reserpine (274).

Tolerance occurs both to the hallucinogenic (167) and pyrogenic actions of lysergic acid diethylamide (240). Rothlin (271) reviews its pharmacodynamics. On the reticular arousal, Bradley (31) suggests that chlorpromazine and lysergic acid diethylamide act in relation to the afferent inflow to the reticular system, whereas amphetamine changes the threshold for direct reticular excitation. Winter & Flataker record (329) a partial antagonism by 5-hydroxytryptamine, and a potentiation by reserpine, of the interference by lysergic acid diethylamide with rat rope-climbing. A very interesting development is the observation that lysergic acid diethylamide can (in high doses) reduce the post-synaptic geniculate response (95), inhibit recruiting from the centrum medianum of the thalamus while specific responses are enhanced (261), and facilitate visual and auditory cortical evoked responses

in low doses (260). The primary facilitation of the auditory response is antagonized by reserpine (260).

Amphetamine.—This substance has often been regarded as primarily a sympathomimetic drug; but it appears that its action on isolated smooth muscle preparations and on the blood pressure is largely "tryptaminomimetic" (314). There is even evidence for some nicotinic action (184). It is noteworthy that Longo (212) found it aroused effectively when epinephrine did not. Amphetamine resembled lysergic acid diethylamide in producing both electrical and behavioral arousal, and could be antagonized in both respects by chlorpromazine (33); and it lowered the threshold to reticular stimulation (31). Paasonen & Vogt (243) showed that amphetamine would reduce brain 5-hydroxytryptamine about 50 per cent. Gaddum & Vogt (127) found that amphetamine intraventricularly produced vomiting and then a lethargy which intensified 5-hydroxytryptamine action by the same route; parenterally, however, it exerted its usual analeptic effect, and antagonized 5-hydroxytryptamine.

Mescaline.—When injected into the ventricles mescaline produces a "catatonic" state in cats and dogs after initial autonomic stimulation (299); the action is fully inhibited by chlorpromazine; reserpine lessens the catatonia; azacyclonal changes the responses so that excitation is more prominent. In high concentration topically it has a strychnine-like action on the cortex and lateral geniculate body (272). Vane (314) finds it to be primarily 5-hydroxytryptamine-like rather than sympathomimetic. The extensive but shaky theory of M-substance in schizophrenia, and its relation to hallucinogens such as mescaline, adrenochrome, adrenolutin, and other compounds, has been reviewed (171).

SUBSTANCE P

Angelucci (8) records a release of a substance that might be Substance P from frog spinal cord. Brain Substance P is unaltered by amphetamine, ephedrine, insulin, β -tetranephthylamine, caffeine, ether, or reserpine (243). Substance P given intravenously in cat does not modify ganglionic transmission or the vascular response to carotid occlusion; intraventricularly a stimulation of respiratory frequency and depth occurs, and a loss of "spontaneity" in the animal's behavior (94). Applied to a perfused rabbit ear isolated save for nervous connections, it provokes a reflex stimulation of respiration and fall of blood pressure; hexamethonium does not prevent this (although it antagonizes the similar response to acetylcholine), but cocaine abolishes the response to P (201). Minz & Walaszek (227) find an increase of Substance P in rabbit brain after treatment with serum from schizophrenics.

INHIBITOR SUBSTANCE OF FLOREY (I)

Brockman & Burson (37) find that glutamic acid and aspartic acid as well as γ -aminobutyric acid can inhibit the opener system of crayfish claw, implying that γ -amino butyric acid (or its derivatives) may not be wholly

responsible for I activity in an extract. I appears in fluid bathing the cortex (112). In the first experiments (112) on its effects, it was only active in acid solutions. Paralysis of inferior mesenteric and stellate ganglia, but not of superior cervical ganglion or of neuromuscular transmission, was produced. Applied to exposed spinal cord (113) it depressed the stretch reflex, but potentiated or even elicited the flexor reflex. An odd feature was the speed with which effects were produced (a few seconds); this is too soon for any reasonable rate of diffusion to the gray matter, and prompts the doubt that the effects were due to a nonspecific effect on surface structures, perhaps temporarily exciting nociceptive afferent fibres. Florey (111) finds a marked accommodation, both to I, the inhibitory substance, and to acetylcholine, by the crayfish stretch-receptor, such that when acetylcholine is washed out a period of silence in the discharge ceases, and when Florey's inhibitory substance I is washed out, the receptor is activated.

An interesting link with this work lies in the convulsant action of semicarbazide and related compounds (186, 187). The convulsions develop only after a latent period of about one hour, and are associated with a reduction of γ -aminobutyric acid in the brain, probably due to inhibition by the semicarbazides of the pyridoxine-dependent enzyme glutamic acid decarboxylase. Pfeiffer and his colleagues (256) find that the convulsions are prevented by pyridoxine and certain anticonvulsants, and that in cats convulsive activity begins first in the aqueductal gray matter and head of the caudate. Semicarbazides also antagonize histaminase (11) and can produce beak and bone deformities in chick embryos (241).

GENERAL PHYSIOLOGY

Spinal cord.—Frank & Fuortes (118) record data on the electrical constants of motoneurons. Membrane depolarization required for excitation is about 1 mV less by synaptic than by direct excitation: accommodation is less prominent than in nerve. Fuortes & Hubel (123) have brought out the role of temporal summation in extensor, as opposed to flexor, reflexes, and Granit *et al.* (141) have used post-tetanic potentiation to distinguish tonic and phasic motoneuron discharges to a stretch of ankle extensors. Lloyd and Lloyd, & Wilson (208, 209, 210), have analyzed in detail the monosynaptic response with repetitive excitation at various rates.

Brooks *et al.* (40) find that bulbar or suprabulbar stimulation may inhibit spinal reflexes without impairing antidromic responses, posttetanic potentiation, or the facilitation of antidromic responses by orthodromic excitation; they conclude that the inhibitory process is postsynaptic. Lindblom & Ottosson (205) find fibres in the pyramidal tract depressing the N_1 dorsum potential (to low threshold skin afferent stimulation). Eccles, Fatt & Landgren (83) have verified that the inhibitory neurones activated by Ia or Ib fibres have the low threshold, specificity, frequency of discharge and lack of subliminal fringe required if they are to act simply as "phase-switches" from excitation to inhibition in muscle reflex paths. Edisen (87) proposes that, if

synaptic excitation in a motoneurone occurs in dendrites and saltates from dendrite to axone, inhibition could be produced if the concomitant soma depolarization increased membrane conductance more than the electrotonus transmitted through soma membrane assisted axonal stimulation.

Frank & Fuortes have followed an earlier paper (116) describing the neural structures encountered when a microelectrode is passed through the cord, by an account of the properties of interneurons (117). Diverse response patterns occur, and antidromic shocks activate some cells (Renshaw cells) but silence others, implying that Renshaw cells play on interneurons as well as motoneurons. Skoglund & Kolmodin (289) have analyzed the convergence on interneurons in the lumbar region. Gelfan & Tarlov (131) describe a drastic but apparently partially reversible method of modifying spinal cord responses, by compressing about 5 mm. length of cord with suprasystolic pressures. McCouch & Austin (215) have further analyzed the N_1 and N_2 waves of the cord potential. Ethyl alcohol depresses mono- and multi-synaptic reflexes equally (192).

In an elegant series of papers, Laporte, Lundberg *et al.* (173, 198, 199, 200, 214) have studied the properties of the dorsal spino-cerebellar tract, using the dissected fascicle. Muscle nerve stimulation yields synchronous volleys, and intra-axonal records show that single shocks produced single spikes; with skin nerves, repetitive discharges occurred with fibres firing up to 16 times per stimulus at frequencies up to 1000/sec. Cerebellar projection was mapped out. Muscle-spindle and Golgi-tendon organ responses were identified, sometimes converging on to the same neurone. Activity in the tract depends partly on interneuronal activity. The transmission process in Clarke's column appears to be very efficient, comparable to that in the cuneate nucleus (302), the intermediate nucleus (83), and perhaps the lateral geniculate (25). Post-tetanic potentiation is present, though small. Yamamoto *et al.* (334) have studied the afferent inflow to the posterior columns.

Despite the vogue for chemical transmission, interest is maintained in the role of slow or steady potentials. Gopfert (139) has restudied the dorsal root potential of frog spinal cord; it is remarkably resistant to asphyxia or storage of the cord in the cold. If the cord substance is almost entirely removed from the root so that only a few neurones can be found histologically, a dorsal root potential of 1 mV can still be recorded with external electrodes. Koketsu (191), using impaled dorsal root fibres in the funiculus of bullfrog isolated cord finds, with a shock subthreshold for the impaled axone, that a slow inside-positive potential is recorded, about 4 mV in magnitude, which is not recorded outside the fibre; he concludes that the central ending of dorsal root fibres is depolarized secondarily by the activity of other dorsal root fibres or interneurons. Wall *et al.* (319) have analyzed excitability changes in terminal arborizations of spinal afferents; for skin afferents, hyperexcitability may persist over 150 msec.

Cortex.—A valuable critical discussion of cortical structure and function

will be found in Sholl's book (285). Beurle (21) has analyzed mathematically the properties of a mass of cells with neuronal properties; such a system would allow the propagation of waves of activity under definite conditions, and furnishes a model for conditioning, learning, and memory.

Spreading depression has received considerable attention. Grafstein (140) has shown that a burst of intense activity, followed by cortical depression, signals the advance of the negative wave: evidence is advanced in support of the suggestion that the depression is transmitted by K^+ release. It is known that spreading depression propagates at 2 to 5 mm/min. as a 10 to 15 mV surface negative wave, followed by surface positivity; it resists brief asphyxia, is abolished by topical cocaine, crosses a cortical cut if the edges are coapted (but not a scar, 157) and resists barbiturates but is reduced by ether. There is a rise of cortical impedance (154) and an increase in local blood flow (296). Species variations exist (156) such that the reaction can only be elicited once in monkey, occasionally in cat, and every 6 to 10 min. in rabbit in whose cortex it spreads widely. As an animal grows, the development of the injury potential recordable from the cortex goes parallel to the capacity for showing spreading depression (48). Metrazol or photic stimulation after metrazol may produce it in the rabbit (155) in which spreading depression may be a protective mechanism.

Burns, Grafstein & Olszewski (53) have shown that the cells responsible for the "burst" response and after-discharge are mostly in layer V, are among the largest neuroses, include but do not only consist of Betz cells, and have connections lying in layers IV and V. Hunter & Ingvar (177) have analyzed the paths (cortical, subcortical, and possibly a third more complex path) by which irradiation from visual to other cortical areas occurs of the response to a light-flash after metrazol. Bernhard and colleagues (18, 19) find lidocaine very effective in controlling cortical seizures, acting at cortical level, without affecting nonconvulsive activity.

Projections from the cortex have been studied (326) including those which affect the N_1 cord dorsum potential (205), or involve the intralaminar thalamic nuclei (239), the dorsomedial thalamus and pons (293), and the brainstem reticulum, pons and medulla (268), or control tegmental conduction (2). The pathway of the sympathetic vasodilator system (distinct from the hypothalamic autonomic system) has been mapped out from the motor regions of the cortex downwards (206). Spatial organization of the corticospinal tract has been found largely to disappear below the internal capsule; the dorsal spinocerebellar tract splits the tract in the cervical region (15).

Projections to the cortex analyzed include thalamic projections from the ventrolateral nucleus to motor cortex (202) which yield responses possibly from cells superficial to pyramidal cells and accompanied by arrest of cortical activity; from posterior ventral nucleus to sensory cortex mediated by a monosynaptic path firing at rates up to 500/sec.; and from medial thalamic regions (203), mediated by a long-latency recruiting path to several cortical

layers. Lesions of ipsilateral ventral posterior thalamic nuclei produce a supersensitivity of sensory cortex to methacholine and metrazol (297). Clare & Bishop (61) contrast the two types of responses to stimulation of lateral dorsal and medial dorsal nuclei: a short latency response unchanged or fading on repetition at 6/sec., and a longer latency response of higher threshold, longer duration, which "recruits." The latter response shows a supernormal phase at 1/6 to 1/8th sec. which the short latency response lacks. They suggest that the fast response may be of small myelinated fibres, the slow response that of nonmyelinated fibres, and that the "recruitment" may be a misnomer for a local increasing activity in particular dendrites. Jasper, Naquet & King (179), stimulating the intralaminar n. centralis lateralis, obtained vigorous recruiting responses over wide cortical areas, including sensory cortex, but point out that failure to find it in sensory cortex may be due to a poor preparation, incorrectly placed stimulating electrodes, or excessive sensory activity. Brookhart & Zanchetti (39) distinguish carefully the "augmenting" and the "recruiting" response (the latter being generalized, slowly developing, with long latency and minimal initial positivity). The "augmenting" response appears to correspond most closely to spontaneous spindle bursts, as judged by their influence on pyramidal discharges.

Kruger (195) has studied the evoked responses in precentral gyrus to somatic afferent volleys (fast cutaneous or slow muscle afferents); these responses do not arise from postcentral or anterior cortex, and survive pyramidal section or cerebellar ablation. The cortical responses to nociceptive stimuli are radically modified by varying depth of anaesthesia (237). The cortical and other responses evoked by light have been analyzed by Bremer & Stoupe (34), Malis & Kruger (217) and Harman & Berry (153) and unitary auditory responses by Erulkar *et al.* (91). Baird & Spiegel (13), Cragg & Hamlyn (70) and Blackstad (26) report work on hippocampal and amygdaloid regions.

Subcortical and mesencephalic structures.—Feldberg (105) has discussed the types of response resulting from intraventricular injections: (a) convulsions corresponding to centrencephalic epileptic seizures, produced by small doses of d-tubocurarine (108); (b) a condition resembling surgical anaesthesia, elicited by epinephrine, norepinephrine and ergotamine; (c) a catatonic state induced by bulbocapnine (109) or anticholinesterases. Although this work has not allowed any definite statement about transmission at any histologically specified synapse, these general reaction patterns are of interest, particularly in conjunction with the distribution of hormones and of diffusely reacting neuronal systems in the periventricular regions. The method has been widely used; it has even been applied with similar results to the mouse (150, 151). The observation that injections of glucose into the cerebral spinal fluid cannot prevent the effects of systemic insulin hypoglycaemia on the spinal cord indicates that drugs so injected can have only a limited penetration (330). Similar centrencephalic seizures to those produced

by d-tubocurarine are elicited by injections of procaine-penicillin into the lateral nucleus of thalamus, putamen, globus pallidus or brain stem (98), and penicillin injections into non-specific thalamic areas evoke synchronized $3\frac{1}{2}$ to 5/sec. cortical spindles (298). Penicillin and strychnine have also been used in analyzing thalamo-caudate-pallidal connections (263). Bulbocapnine has been tried for sedation of children for electroencephalogram recording (12). Fukuma (122) reports on the central effects of veratramine. Gangloff & Monnier (128), from a comparison of the effect of various anti-convulsants on excitability of diencephalon, rhinencephalon, and cortex concludes that diencephalic action by such drugs confers protection to *grand mal*, and diencephalic and cortical action is required to protect against *petit mal*.

Parma & Zanchetti (245) find that judicious reticular stimulation can selectively depress the surface negative phase of the augmenting response to slow thalamic excitation; the fact that pyramidal discharge still accompanies the positive phase confirms the association of these two. Anaesthetics rapidly produce cortical desynchronization (an effect removed by mesencephalic section) and abolish strychnine's local cortical effects (269). Chloralose activates the human electroencephalogram (231). Wycis *et al.* (333) have analyzed the tremor produced by tegmental stimulation. Scheibel *et al.* (276) describe the extensive convergence of diverse afferent pathways on the reticular system. Papez gives an account of the central reticular pathways (244). It is claimed that reticular excitation can influence the state of consciousness in monkeys (258). An interesting study by King (188) shows that barbiturates strongly depress arousal, depress reticular response to sensory stimulation, favor recruitment, and leave cortical responses to sensory stimulation unchanged. Mephesisin, on the other hand, does not influence arousal, but depresses recruitment, sensory cortical responses and reticular responses to sensory stimulation. Mephesisin can remove the enhanced recruitment induced by barbiturates but not the depression of arousal; but barbiturates can restore recruitment depressed by mephesisin. This seems to imply that mephesisin does not attack recruitment directly, but depresses some facilitating mechanism, the loss of which can be offset by the withdrawal (under barbiturates) of desynchronizing reticular influences. Sharpless & Jasper (282) have analyzed habituation of the arousal reaction, distinguished two types of arousal, and discussed the pathways involved.

Responses to intravenous beta-hypophamine (Pitressin) (but not to ephedrine or epinephrine) in the region of the mammillary bodies are described (300). Injections of hypertonic solutions led to electroencephalogram changes, and if repeated, to seizures in amygdala or hippocampus, implying that osmoreception may be more extensively distributed than the preoptic region. Purpura (259), by cross-circulation experiments, has found stimulation of bulbar reticulum of one animal to lead to humoral activation of the recipient's cortex. It is hard to believe that a reticular transmitter should be detectable after crossing two blood-brain barriers and mixture with a

large amount of blood, but a generalized release of reticular hormones might be responsible.

Bishop & Evans (25), studying the optic tract synapse in the lateral geniculate body, find the postsynaptic response supernormal while the tract is still partially refractory; postsynaptic response varies linearly with tract activity, and brief discharge rates up to 1000/sec. are seen. With continued excitation, steady potentials (soma region as sink) can be recorded (315). Post-tetanic potentiation can be shown as well as post-tetanic subnormality (95, 176); prolonged tetani can remove the subnormality resulting from briefer tetani (96). The effects of mescaline and lysergic acid diethylamide have been tested (272). Unitary responses in the medial geniculate body and the effect of morphine have been analyzed (316).

Cerebellum.—Granit & Phillips (143, 144) have made a fascinating study of unit responses by Purkinje cells, deserving extensive discussion. Prepotentials (often at rates of 200/sec.) were recognized, attributed to the massive dendritic input to a Substance P-cell, but no "generator potential." Two kinds of inhibition were found, one apparently a cathodal depression (attributed to basket cell activation) and usually occurring spontaneously, the other probably hyperpolarizing, and elicitable by stimulation. Grant, Holmgren & Merton (142) have shown that cooling or removing the anterior lobe of the cerebellum in the cat "switches" the response to ear-twisting from γ to α pathways; they suggest that this part of the cerebellum determines the body's choice between movements controlling muscle-length (plastic in character and dorsal-root-dependent) and movements controlling muscle-tension (nonplastic and independent of afferents). The characteristic loss of length (position) control in patients with cerebellar lesions conforms well with the suggestion. Evidence as to the role of γ -fibres comes also from the experiments by Matthews & Rushworth (221, 222) on the effect of procaine (which paralyzes γ -fibre activity first) on stretch reflexes. γ -fibres are also particularly sensitive to x-irradiation (132). Projections to the cerebellum from splanchnic afferents (327) and dorsal spinocerebellar tract (228, 229) and from the deep nuclei (55) have been examined, as well as the mechanism of the crossed disappearance of extensor rigidity following caudofastigial lesions (230). X-irradiation (4) and DDT (2-2 bis (parachlorophenyl) (1-1-1 trichloroethane)) (242) produce interesting specific cerebellar lesions.

Area postrema.—Brizzee (35) finds that thermal coagulation of the region in monkey abolishes the emesis after x-irradiation, although a similar lesion to the nearby dorsal vagal nucleus had no effect. Clemente & van Breeman (64) have shown the area to be full of fine unmyelinated fibres, and some thicker ones, entering from its lateral aspect, terminating on the cells there or on blood vessels. Clemente, Sutin & Silverstone (65) have also shown that intravenous injections of hypertonic saline lead to increased nervous activity in this area.

EFFECTS OF CERTAIN DRUGS

Strychnine.—Eccles and his colleagues (69, 74, 82) have established that strychnine depresses or abolishes inhibitory postsynaptic potentials but not excitatory postsynaptic potentials. Its interference with Renshaw cell effects is exclusively at the Renshaw axon endings. The question arises as to why strychnine is so ineffective on some types of inhibition, such as the inhibition of Head's diaphragmatic slip by lung inflation (Creed & Hertz, 72). Bradley, Easton & Eccles justly point out (32) that in a polysynaptic inhibitory reflex, removal of inhibition at intermediate relays might intensify the final discharge; but it is hard to imagine that it would be so intensified as to restore to normal the final postsynaptic response, in the presence of enough strychnine to paralyze another inhibitory synapse completely. An obvious possibility is that there is a second, strychnine-resistant, inhibitor.

Cobb *et al.* have analyzed (66, 67) the spike response to photic stimulation after strychnine, and find spike activity to be transmitted along neuronal chains in the deeper cortical layers. Terzuolo & Gernandt (301) have correlated unit discharges with spinal cord potentials during strychnine tetanus. Clare & Bishop (62) used strychnine to poison cortical dendrites.

d-Tubocurarine.—This drug continues to be used by intravenous route without adequate control. Its ability to release histamine, for which it is a standard drug (see (249) for references), is a serious complication only to be controlled by use of an equiactive histamine-liberator, or by previous exhaustion of releasable histamine; records of blood pressure or comparisons with injected histamine or epinephrine, do not allow an adequate estimate of the effects of the release. The ability of heparin to antagonize apparent central actions of d-tubocurarine (262) almost certainly rests on complex-formations between the two drugs (249). The ability of d-tubocurarine to paralyze ganglia and the γ -endplates must also be considered.

Tetanus and Botulinum toxin.—Brooks, Curtis & Eccles (42) establish clearly that tetanus toxin resembles strychnine in antagonizing the production of inhibitory postsynaptic potentials while being inactive on excitatory postsynaptic potentials, or on the cholinergic Renshaw cell synapse. This does not altogether agree with the known pharmacology of tetanus toxin [confused though it is (332)], for which a cholinergic mechanism of one sort or another has usually been envisaged (6, 159). Botulinum toxin, tested on miniature end plate potentials reduces their frequency but not their size (41), and can be antagonized by post-tetanic potentiation.

Morphine.—The actions of morphine and morphine-substitutes on guinea pig ileum show a striking parallelism with their activity centrally. This lends some importance to the finding that low concentrations of morphine depress acetylcholine output by the gut, both in the resting state (253, 275a) and when its nervous tissue is excited (253). It is even possible to produce "tolerance" and "dependence" in the ileum *in vitro* (253).

Tranquillizing drugs.—The rapid multiplication of these drugs has not simplified the pharmacology of the central nervous system. The classification (17) as (a) phenothiazine derivatives, e.g., chlorpromazine; (b) reserpine-like; (c) diphenylmethanes; (d) substituted propamidiols, is helpful, but other structures are important. (The following references to these and related agents, may be noted: 14, 17, 24, 31, 33, 43, 129, 165, 166, 175, 180, 219, 238, 255, 257, 264, 275, 279, 287, 299, 321).

BLOOD-BRAIN BARRIER

A major obstacle to pharmacological study is the existence of the so-called blood-brain barrier. Feldberg, Malcolm & Sherwood (108) and Eccles *et al.* (74, 82) have encountered again the well-known failure of quaternary or strongly water soluble substances to penetrate the central nervous system [cf. Burgen & Chipman (50)]. The heroic experiment by Smith *et al.* (291), in which a conscious subject received three times the respiratory paralyzing dose of d-tubocurarine, but retained mental function and memory completely unimpaired, remains a classical demonstration of the failure of the drug to produce central actions. The observation (248) that cerebrospinal fluid levels of hexamethonium may be less than one per cent of the plasma level, even after a lapse of 1 to 2 hr., emphasizes the same point. Mayer & Bain (224) have attempted to localize the barrier with fluorescent quaternary compounds, and place it in the region of the outer wall of the capillary endothelium and glial plasma membrane. The barrier is present (145) in 12 day rat embryos, when neither astrocytes nor Hess' ground substance are developed. Koelle & Steiner (190) compared the reduction of brain cholinesterase achieved by parenteral and intraventricular injections of two phosphorous antiesterases, one quaternary, one tertiary; parenterally the quaternary had negligible action, although 1/100th dose was strongly effective intraventricularly. Evidence of the simultaneous presence of the quaternary compound and active acetylcholinesterase in brain tissue was obtained, and is regarded as a possible model for the occurrence of acetylcholine and cholinesterase within a single cell. CO₂ inhalation (10 per cent or more) increases the penetration of trypan blue into rabbit brain, but the effect ceases with the inhalation (63).

AUTONOMIC GANGLIA

R. M. Eccles (85) has reported her experiments with intracellular electrodes. Resting potentials of 65 to 75 mV were seen. Spikes were up to 90 mV, of 4 to 7 msec. duration with an inflection (identified as synaptic potential) on the rising phase, and followed sometimes by an afternegativity of 1 to 5 mV preceding the usual slow positivity. Synaptic potentials, giving rise to a spike at about 15 mV, lasted about 60 msec. in the absence of a spike. In later experiments (cited J. C. Eccles, 81) antidromic spikes were recorded, with the "IS-SD" transition, "SD" responses sometimes failing to appear. A subsequent paper (86), in which external electrodes were used

as in earlier studies (84), describes the effect of nicotine on isolated rabbit ganglia. The ganglion depolarization and change in after-potentials described by Paton & Perry (247) are confirmed; it was found in addition, that with repetitive stimulation, nicotine led to more marked afterpositivity, and that nicotine reduces the synaptic potential obtained under dihydro- β -erythroidine (sometimes after temporarily restoring a spike) and the "late negative" wave.

Pascoe (246), on a similar preparation, describes depolarization produced by acetylcholine in the presence of an antiesterase, by tetramethylammonium salt or nicotine, which is followed by a large positivity on washing out. The depolarization is insensitive to temperature but the positivity is increased by moderate cooling (maximum at 30°C.) and almost disappears at 2 to 3°C. or 40°C. Hexamethonium reduced both effects evenly, and behaved like a competitive blocking agent, but d-tubocurarine removed the positivity selectively.

Hutter & Kostial (178) find that although perfusion of a ganglion with Ca-deficient Ringer produces block, perfusion with isotonic NaCl does not. It appears that K is needed to bring out the effect of Ca lack on the ganglion. Gertner & Reinert (133) conclude that K_i changes are not responsible for the reactions of a denervated ganglion, but that losses of amino-acids are more likely. Trendelenburg (306, 307, 308) has obtained further evidence that 5-hydroxytryptamine, like histamine and pilocarpine, produce a stimulant action resistant to hexamethonium and d-tubocurarine, but abolished by cocaine and during the first phase of nicotine's ganglionic action. He also finds (309) a central stimulant effect of histamine by ventricular injection. Malmajac (218) has studied the splanchnic-suprarenal medulla "synapse," finding that small doses of epinephrine facilitate and large doses depress transmission. Matthews (223), on the other hand, finds epinephrine and norepinephrine in small doses only depressant on the cat superior cervical ganglion, although isoprenaline augmented submaximal responses.

Recent work on ionic fluxes and peripheral nerve promise to illuminate ganglionic responses. Thus ouabain reduces Na efflux and K influx in red cells (138, 294); low K_0 has a similar action; and denervation of a muscle reduces K influx (158). It is interesting that ouabain and denervation sensitize the ganglion to acetylcholine, and low K_0 or denervation bring out a ganglion-stimulant action by hexamethonium. It may well be that specific membrane changes underly these effects rather than the metabolic alterations previously postulated.

There is also a striking analogy between the hyperpolarization and spike augmentation after repetitive stimulation of C-fibres and of ganglia; the afterpositivity studied by Pascoe may be related. The glial cloak to the ganglion cell and its processes has already been invoked to account for some of the differences between ganglionic and neuromuscular potential changes (248a), and a parallelism is obvious to the diffusion barrier postulated by Ritchie & Straub (266, 267). The dendrites of the ganglion, embedded in

their glial envelope, could then be the seat of a positivity due to sodium pump activation and K_0 depletion in the spaces around the fibre. On the other hand, when the ganglion is partly blocked (so that, perhaps, predominantly soma response is seen), late negative waves are more prominent; here the situation may be more analogous to a large diameter structure such as the squid axone, in which the barrier to diffusion near its surface raises K_0 sufficiently to cause a negative afterpotential.

COLLATERAL SPROUTING

Murray & Thompson (233) have found that the return of function after partial preganglionic denervation (288) is due to the development of sprouts from the remaining intact preganglionic fibres. The response is remarkably prompt (sprouts are visible histologically in a few days) and effective (10 per cent of fibres will suffice to restore function completely). Supersensitivity appears during the early stages of the denervation, but disappears as reinnervation by sprouts becomes complete (at about 30 days). The sprout synapses are not fully normal, as acetylcholine output is a little below normal, and they are more than normally sensitive to blocking agents. A re-routing of autonomic pathways occurs, as preganglionic fibres come to impinge on cells previously innervated by other fibres. The process is comparable with that known to occur with sensory and motor fibres, and in the central nervous system, but seems to be more rapid and effective. It has important implications for any attempted autonomic denervation (bearing in mind the inaccessibility of the accessory ganglia), for sequelae of surgical sympathectomy such as gustatory sweating, for disease processes which produce partial denervations, and for certain classical phenomena such as the Sherrington, Phillipeaux-Vulpian, and Rogowicz pseudomotor reactions.

CONCLUSION

It is disappointing that, despite the advances made, we still cannot be confident in the specific identification of any central transmitter save acetylcholine. The processes by which the first sensory neurone transmits activity, or by which the motoneurone is excited or inhibited, let alone the other more complex excitations and inhibitions in higher centres, are still uncertain. Pharmacological study might be facilitated by using synapses with a large safety factor for transmission (on the assumption that a correspondingly vigorous release of transmitter would occur), by the discovery of ways of circumventing the blood-brain barrier, by further development of methods of administering, collecting, and assaying minute quantities of active substances, and by the more certain identification of the origins of some of the bioelectrical potentials studied. But the convergence of so much electrophysiological and pharmacological activity encourages hope that it will not be long before our knowledge of the chemistry of central transmission rises above its present tantalizingly subliminal level.

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SOMATIC FUNCTIONS OF THE NERVOUS SYSTEM^{1,2}

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INTRODUCTION

Over any period of a few years, new ideas make their appearance on the neurophysiological scene, are tested experimentally, and usually provoke controversies calling for new tests and the development of new methods. Rarely, a successful one becomes an integral part of current thinking about the function of the nervous system. This year is marked by the maturity of several new ideas. G. H. Bishop's review describes the first of these, that explosive conduction is only one of the modes of activity of nerve cells (23). Both dendrites and telodendrites are capable of graded more-or-less, rather than all-or-none, activity. This shows no refractory period, can be summed and extended over long periods of time, occurs in either excitatory or inhibitory directions, and continually conditions the discharge of conducted impulses. The cellular mechanisms underlying the central states of Sherrington seem finally revealed, appropriately so in his centennial year.

A simple, clear, and well documented hypothesis of the integrative action of motoneurons is described by its principal architect, J. C. Eccles, in his book *The Physiology of Nerve Cells* (96). It rests on the finding that the initial segment, the axon hillock, is a region of the nerve cell of high excitability, continually played upon by synaptically evoked excitatory and inhibitory responses of soma and dendrites; the temporal pattern of motoneuron firing represents the integrated sum of these two processes.

For several years a trend in the use of electrophysiological methods in the study of central nervous function has been clear. While these methods continue to reveal new and more perfect data on the location of tracts, nuclei, and cortical areas, they more and more are used to study the spatial and temporal patterning of neural activity of one particular type or another, set amidst the ever oscillating spontaneous workings of the nervous system: the question asked is how as well as where. In particular, the application of Adrian's method of single unit analysis to many fundamental problems at all levels is increasingly productive of new facts which support old and are used to create new hypotheses. These subjects have recently been subjected to a comprehensive review by Albe-Fessard (4).

The application of electron microscopy in study of central nervous histology reveals a wealth of new information, in particular the presence of myriads of very fine fibers entwined in the neuropil. It seems quite obvious

¹ The review of literature pertaining to this review was completed in June, 1957.

² The following abbreviations are used in this chapter: p.d. (potential difference; e.p.p. (end plate potential).

that so far microelectrode studies have selected only larger elements, which make up only a small percentage of the total. A method for studying the action of unmyelinated fibers in the nervous system is badly needed.

The study of activity levels and the systems which control them goes forward on a broad front, but it is not yet possible to set what is known of the so-called reticular activating and diffuse thalamic systems together in a unified and parsimonious theory.

The papers reviewed below make up no more than 25 per cent of those published in the field during the year. The selection reveals nothing but the interests of the reviewer.

SPECIFIC AFFERENT SYSTEMS

THE SOMATIC AFFERENT SYSTEM

Receptors.—Bullock & Diecke (43) report the results of an extensive study of the facial pit of pit vipers; its receptors are true temperature detectors sensitive to radiant heat in the spectrum range 1.5 to 15 μ . The organ detects not only warmth by radiation but also its direction and whether it is moving, an adaptation of great importance to the species. The receptors show a fluctuating spontaneous activity which provides for high sensitivity and a capacity for signalling both positive and negative changes. Both here and in another paper Bullock provides a lucid discussion of the problems for central integration posed by this spontaneous activity, which has now been observed in many and diverse receptor types: how are real signals recognized against such a fluctuating background of input (42) [see also (158), (12)]. FitzHugh has considered this problem also in his statistical analysis of the informative significance of the response of single ganglion cells of the cat's retina to brief stimuli, contrasted with their spontaneous level of discharge (106).

Frog's skin contains mechanoreceptors, some of which show a rapid and others a slow rate of adaptation [Loewenstein (179)]. The first group responds to steady stimuli with on and off discharges when the skin is loose, but at high degrees of applied tension of the skin assumes the properties of the second group, which shows a dynamic on component, steady state discharge during steady stimulation, and release depression. The hypothesis is presented that the rate of adaptation of a nerve ending depends upon the rate of decline of its generator potential, which in turn is determined by the degree of laxity of the terminal nerve membrane and its relation to the elastic components of the tissue arranged in series, i.e., the rate of adaptation depends upon mechanical factors and not upon qualitative differences in receptors. Loewenstein reports also that the fast-adapting receptor is facilitated by application of epinephrine or by stimulation of the sympathetic efferents to the skin, while the stretch receptor is not, but whether this is a direct effect upon receptor sensitivity is not clear (178).

Diamond, Gray & Sato present further evidence that the pacinian corpuscle responds to mechanical stimuli with a generator potential, graded by strength of stimulus, which may reach the level of the membrane potential,

and that no all-or-nothing activity occurs in the distal unmyelinated segment of the innervating nerve fiber. Depolarization of the contiguous node of Ranvier by the generator potential elicits conducted action potentials. The terminal segment is capable of graded activity only (90). Gray has reviewed the general subject of the initiation of nerve impulses in mechanical receptors (129), and Pease & Quilliam report a study of the ultrastructure of this receptor (212).

A valuable study of the histology, nerve supply, and endings of Meissner's corpuscles of the human skin has appeared [Cauna (59, 60)]. These highly organized multicellular structures are innervated by two to nine thick medullated fibers, and in some cases by additional fine nonmedullated fibers which are not sympathetic. Skoglund (236) has given a detailed description of the anatomy and physiological properties of another organized ending, those of the knee joint of the cat. Each joint capsule mechanoreceptor subtends an angle of about 15 degrees of joint movement, adapts only slowly when the joint is held in its excitatory angle, and responds phasically as the joint passes through that angle.

Lele & Weddell (168) find that a wide range of sensory experiences can be evoked in man by a suitable variety of stimuli applied to the center of the cornea. This fact, taken in conjunction with clear histological evidence that only free nerve terminals are found in the cornea, is used by the authors as support for the idea that the modalities of common sensibility cannot be equated with four morphologically distinct kinds of peripheral nerve terminations. While this seems reasonable it yields no evidence to question the specificity of the central action of afferent nerve fibers. What has been shown is that this specificity does not depend upon specific types of encapsulated nerve endings, in all skin areas.

Stretch receptors.—The histological detail of the spindle system of frog muscle has been described by E. G. Gray (128). Slow or "tonic" muscle fibers, analogous to the intrafusal fibers of mammalian spindles, are scattered among the fast or twitch fibers. Of the former, some are innervated by small motor fibers (gamma efferents), while others receive the terminals of large motor fibers (alpha efferents). Taking advantage of their accessibility in frog muscle, Burke & Ginsborg have studied the electrical properties of the slow fibers, and the action of the gamma efferents upon them (44, 45). Depolarization to zero p.d. or beyond does not evoke conducted action potentials; thus the failure of impulses in gamma efferents to elicit propagated responses reflects this "inexcitability" of the cell membrane, and a delayed rectification explains the diphasic decay of the e.p.p. produced by transmitter action [see also (159) and (160)]. Transmitter action reduces membrane resistance and drives membrane p.d. to a new equilibrium level intermediate between that of a " K^+ or Cl^- selective" and a " Na^+ selective" membrane, a fact indicating that a change in permeability of the active junctional region occurs to more than one ionic species. Such differential properties of intra- and extrafusal fibers may allow differential blocking of the gamma efferent-

intrafusal junction, which would open a possible means of therapy of spastic states.

Continuing his studies of the gamma efferent system in mammals and its central nervous control, Granit, with Henatsch, shows that efferent impulses in gamma motoneurons exert a strong indirect influence upon the dynamic component of the spindle response, rendering the gamma biased sense organ a faster and more efficient mechanism than the unbiased one (123). Cooper & Daniel show the effect of tonic gamma activity upon spindle receptors of the goat's extrinsic eye muscle, and emphasize the capacity of these receptors to record the rate of application as well as the steady state of stretch (77).

Projection pathways of the spinal cord.—Fast cutaneous afferents ascending in the region of the ipsilateral dorsal spinocerebellar tract of the cat activate cells of the nucleus cervicalis lateralis [Catalano & Lamarche (58)]. From this nucleus fibers decussate between the levels of C₁ and C₂ to the opposite ventral quadrant and reach the contralateral medial lemniscus. Thus a clear explanation is given for the persistence in this species of transmission via fast cutaneous afferents to contralateral sensory cortex after section of the dorsal columns. This persistence occurs also in monkeys, in which this nucleus is much reduced (it is said to be absent in man), and evidence is given by Gardner & Morin that volleys in fast cutaneous afferents project bilaterally into the spinothalamic tracts in primates (116). The authors believe that multiple ascending pathways exist for cutaneous modalities, and that spinal tracts are not modality specific. On the other hand, Collins & Randt have shown that the dorsal column response to hind leg skin nerve stimulation is related to the larger myelinated fibers of the A group, while the more slowly conducting gamma-delta groups project mainly into the anterolateral ascending tracts, although other projections are not excluded (71). The spino-olivary projection is mainly crossed, ascends in the ventral funiculus of the cord, and derives only from cutaneous nerves, mainly from their gamma-delta components [Morin, Lamarche & Ostrowski (193)].

A thorough study of the functional organization of the dorsal spinocerebellar tract (DSCT) has been continued by the addition of two papers to the three reviewed last year from the Lund laboratory [Lundberg & Oscarsson (180); Holmqvist, Lundberg & Oscarsson (143)]. Intra-axonal recording from DSCT neurons indicates that about two-thirds discharge to I-a volleys from ipsilateral muscle nerves, are driven by stretch of muscle, and are silent during muscle contraction; they are related to spindle receptors. About one-third are activated by I-b volleys, are driven by muscle stretch, and are accelerated by muscle contraction; they are related to Golgi tendon organs. An occasional DSCT neuron was activated from either source. Excitatory convergence occurs from a limited field of muscle synergists, while a wider convergence of inhibition from antagonistic muscles was found. Such a wide overlap is shown also by anatomical studies [Liu (174)]. The

low frequency spontaneous activity of DSCT neurons survived dorsal root sections and is attributed to spinal interneuronal activity; spinal interneurons have been shown to project upon the cells of Clarke's column (241). An extensive single unit analysis of the activity of spinal interneurons driven by stretch is reported by Kolmodin, who emphasizes their integrative capacities (156). Axons in the region of Flechsig's fasciculus driven by slow muscle and cutaneous afferents show wide receptive fields, extensive interaction, and prolonged repetitive discharge to single afferent volleys (180, 143). They are thought to make up another projection system, perhaps that studied by Catalano & Lamarche (58).

A similar analysis of neurons of the ventral spinocerebellar tract (VSCT) has been published by Oscarrson (207). Mass discharge in VSCT is evoked by stimulation of the contralateral muscle nerves, but not from skin or ipsilateral muscle nerves. The input appears to be restricted to I-b afferents (tendon organs). Transmission from them to VSCT neurons is monosynaptic. VSCT is completely crossed in the cord, but recrosses in the cerebellar commissure, providing ipsilateral input to cerebellar cortex.

Landau has analyzed the potentials evoked in the medulla by stimulation of peripheral nerve and sensorimotor cortex (163). His results indicate that potential waves recorded in medullary pyramids evoked by afferent nerve stimulation are generated by passing volleys in lemniscal fibers, and recorded in the pyramid through volume. This, added to previous contrary anatomical evidence (119, 192), seems to dispose of the earlier suggestion that the pyramidal tract should be considered an afferent as well as an efferent system (34, 38).

Brain stem and diencephalic relays.—An increasing interest in interactions between afferent and efferent systems at the brain stem level has spurred new study of the structure of this region. Several anatomical papers suggest that the sensory cranial nerve nuclei are likely to be sites of interactions. Rossi & Brodal (228) working in the cat and Torvik (243) in the rat show that a spinal system ascending in the ventrolateral columns projects upon cells of the trigeminal complex, and upon the nucleus of the solitary tract (228, 43). In a complementary paper Brodal, Szabo & Torvik describe the projection of a system of cortical origin upon these same nuclei, the fibers of which arise from widespread parts of the cortex, but most heavily from the frontoparietal regions (37).

Lindblom & Ottoson find that volleys confined to descending pyramidal fibers interact with afferent fibers at the cord level by depressing the N_1 cord dorsum potential evoked by the latter (173). This is interpreted to indicate descending pyramidal control of afferent transmission through cord interneurons—presumably an action upon relay at the segmental level into the spinothalamic system. In this connection, Chambers & Liu report that, using the Nauta technique to reveal degenerating terminals, removals of sensorimotor cortical areas in the cat result in degenerations of descending fibers terminating in the dorsal column nuclei and in Clarke's nucleus of the

cord (61). It appears from these and many other studies that the central nervous system's control of its own input is a matter of considerable importance deserving of the extensive investigation it will now undoubtedly receive, particularly it is hoped by the application of the method of single unit analysis.

Thalamic relay.—Monnier has studied five human cases in which the ventral thalamic nuclei were stimulated via stereotactically placed electrodes, in the course of thalamotomy for incurable pain (191). The subsequent death of these patients was followed by a careful anatomical study of the brains, and location of the sites stimulated. Stimulation of the medial and lateral ventral posterior nuclei (VPM and VPL) produced sensations referred to bodily parts in topographic patterns similar to those found in animals. The author emphasizes that the sensations produced by thalamic stimulation were referred only to the contralateral side of the body. Stimulation of the medial edge of VPM produced the sensation of pain in the contralateral face and mouth.

Torvik, experimenting with the cat and Carpenter with the monkey, find the dorsal ascending trigeminal tract to arise bilaterally from the main sensory nucleus of the fifth nerve and to project upon the medial portion of thalamic VPM, a fact accounting for the bilateral representation of peri- and intraoral structures in thalamic and cortical tactile representations (245, 56).

The effect of changes in central excitatory state and of barbiturates upon transmission through the ventral nuclear complex has been studied (154). Reticular activation shortens latency of the thalamic response to a lemniscal volley, and, peculiarly, eliminates oscillating facilitation peaks from the recovery cycle. Reticular lesions producing EEG signs of coma, however, introduce such facilitation peaks and shorten relatively unresponsive times. The data indicate that activity in the ascending reticular activating system conditions synaptic transmission through this thalamic relay nucleus.

The retrograde degenerations occurring in the ventral thalamic mass after a variety of cortical lesions in 48 monkey hemispheres have been described by Chow & Pribram (66). Though the authors adopted more or less gross anatomical landmarks rather than cytoarchitecture as guides to individual thalamic nuclei, their results are in general agreement with those of earlier workers that the anteroposterior axis of the entire nuclear mass corresponds to the anteroposterior dimension of the cortex. They conclude also that the cells along the dorsoventral axis of a nucleus "terminate on a focal aggregate in the cortex," since they believe this axis remains undifferentiated in the projection. This is in sharp disagreement with the results of evoked potential studies of the pattern of projection into the tactile thalamic region, VPM & VPL. The cells along the dorsoventral axis of VPL are related to peripheral receptive fields distributed successively from axial to apical bodily parts, and project therefore to cortical foci distributed from posterior to anterior across the precentral gyrus (200). There is, however, an undifferentiated axis of this nucleus, which is directed anteroposteriorly,

curving slightly concave medially, and this axis does project upon a focal neuronal aggregate in the cortex.

Somatic sensory cortex.—The value of the comparative approach to the problem of the interrelation of structure and function in the central nervous system is emphasized again by three studies in which the evoked potential method is used to map cortical regions. The porcupine takes his appropriate place in the phylogenetic trend between rat and more complex mammals by virtue of a complete mapping of visual, auditory, and somatic sensory areas [Lende & Woolsey (169)]. It is emphasized here, as in a similar survey of the somatic sensory area of the dog, that the second area assumes no mean position among cortical hierarchies [Pinto Hamuy, Bromiley, & Woolsey (217)]. Somatotopic localization within it is as exquisite as in area I. In the semilissencephalic squirrel monkey also, somatic II possesses a somatotopic specialization equivalent to that of the postcentral area [Benjamin & Welker (20)]. By an admirable correlation of mapping data with the cytoarchitecture of the brains studied these authors show that the somatotopic pattern of area I in the postcentral gyrus extends anteriorly from classical koniocortex, across intervening transitional fields, to overlap to a considerable extent upon the gigantopyramidal motor cortex. From the published figurine maps it appears that only the representation of apical portions of the extremities overlap the motor pattern, which in this locale is devoted also to apical tissues. Whether this represents another and independent cortical representation of somatic sensibility in the motor cortex is left open by the authors. Kruger poses the paradox that electrically induced synchronous volleys in afferent nerves evoke responses in the motor cortex in the macaque monkey, while no form of physiological stimulation delivered to tissues innervated by those nerves does so, either in anesthetized or unanesthetized animals (157).

The cortical evoked potential in somatic sensory areas has received little attention. Landau & Clare describe the cortical response evoked by electrical stimulation of ventral posterior nuclei (164). Such a stimulus evokes a polyspike response similar to that studied by several authors in the visual cortex (see below), and this type of response seems a typical property of primary sensory cortices. Towe has contributed an exceedingly valuable review of the whole subject of evoked potentials in the somatosensory cortex (246).

General properties of somatic sensibility.—In his Maudsley lecture Brain (29) provides a thoughtful discussion of some of the problems of perception and the mind-brain relation, and Critchley's review (79) of the subject of corporeal awareness is an amusing and critical discussion of much the same subject. These two philosophically elegant papers make delightful reading for neurophysiologists interested in the fields of sensation and perception.

Several investigations of somatic sensibility carried out in waking humans are of interest and importance. Marshall shows that the capacity to localize is considerably better for tactile than for painful stimuli (183). Jones considers the proposition that space and time may be interchangeable to

some degree in central nervous activities, and that increasing the temporal separation of two cutaneous stimuli, for example, should decrease the spatial separation required for them to be recognized as arising from different places (149). She found results in several subjects to fit this notion, but the weight of evidence from single unit studies of the somatic afferent system is heavily against it. Kester has measured the efficiency of various tactile surfaces (153). As organs, the fingernail or an instrument held lightly in the palm proved superior to the tip of the finger pad or the tip of the tongue in recognizing fine scratches on wood, iron, or glass, findings which strongly suggest the role of periosteal receptors in fine discriminations. Spatial alternations of the receptor sheet facilitate perception of very fine scratches, especially if the test surface is bordered by a small wall against which the finger can oscillate. Under these circumstances scratches on iron measuring 1μ across can be detected with certainty. Evidently the tactile system possesses a discriminatory capacity of the same order as that of the auditory or visual.

THE AUDITORY SYSTEM

A masterly review of the biophysics and physiology of the inner ear by Davis (81) has appeared since Tasaki's review of the general subject last year. A fairly satisfactory account of the dynamics of the cochlear partition can now be given: a pattern of traveling waves is formed upon it which leads to a final critical event, the bending of the hairs of the sensory cells. The electrical events occurring within the cochlea can be related with some certainty to the mechanical events, but the origin of the electrical events remains uncertain. A duplex theory of cochlear function is reported to be gaining favor. The volley principle of Wever is valid at low frequencies, but it is equally certain that individual nerve fibers have definite upper frequency limits, associated with the position of the traveling wave pattern upon the cochlear partition, which is determined by frequency. Thus the place principle for coding of frequency is also valid. The problem of frequency discrimination in hearing remains, for the response region of individual nerve fibers, though sharp at its upper end, is otherwise broad, especially as intensity rises. Further sharpening must occur, perhaps by inhibitory action at the first relay, within the cochlear nuclear complex. This general synthesis of theories of pitch discrimination is further detailed by von Békésy, who emphasizes that the traveling waves on the cochlear partition have flat maxima, and that, if the mechanical disturbance is above threshold throughout this flat maximum, further sharpening must occur in the central relays (17). The functional meaning of the different terminations of the spiral and radial fibers of the cochlear nerve is unknown, but if the standing wave idea is valid, cochlear nerve fibers should fall into two different groups, the first responsive to narrow and the second to broad frequency ranges. Such a division has not been observed, and more extensive single unit studies of the first order neurons are badly needed.

The study by von Békésy of a model of cochlea in which the skin of the

arm serves as sensory receptor sheet is of great interest, not only as model of cochlea but as a method for studying the discriminatory capacity of the somatic afferent system as well (18). DeRobertis has studied by electron microscopy the terminals of first order neurons in the cochlear nuclei in both the normal state and at intervals after acoustic nerve section. Degenerative changes and loss of synaptic vesicles are well advanced as early as 22 hr. after nerve section (86).

In a beautifully controlled series of experiments Galambos has shown that efferent impulses in the olivocochlear bundle reduce or abolish the auditory nerve discharges evoked by clicks (115). This effect is completely independent of action of the stapedius muscle, for the phenomenon persists after removal of the bones of the middle ear, and in the curarized animal. It is permanently lost when the olivocochlear bundle is severed peripheral to the site of stimulation. This central efferent pathway has the capacity to modulate afferent input to the auditory system.

Whitfield reviews the electrophysiology of the central auditory pathways, emphasizing the transformation in the spatiotemporal pattern of activity with succeeding relays in the system, which "concentrates" the frequency and intensity aspects of the stimulus into particular neural channels (256). Preliminary reports of Japanese workers indicate just such a narrowing of the frequency response bands of single elements at successively higher levels (151, 240). One possible mechanism for such narrowing may be that observed by Vernier & Galambos in the medial geniculate of the cat (251). Here most single neurons respond one-to-one at low stimulus rates, but as frequency of stimulus increases some elements respond only to the first stimulus of a train, and then fail, while others equilibrate at moderate rates of discharge. Thus increasing the intensity of the stimulus, i.e., frequency of neural input, may cause a narrowing of discharge zone at the thalamic level.

In a preliminary note Erulkar describes the sensitive relation of cells of the inferior colliculus of cat to the intensity of clicks driving them (99). Binaural interaction upon single elements was observed. Response to clicks was abolished by pure tones within a well-defined region of the frequency spectrum for each unit, while some neurons displayed quite narrow frequency response bands as well. The method of single unit analysis has now been applied to the auditory cortex of the cat. Limiting their observations to the posterior segment of the first auditory field, Erulkar, Rose & Davies found cells responsive to sound in layers II through VI of the cortex, though under general anesthesia a large proportion of the spontaneously active cells could not be driven by sound stimuli (100). Some neurons were observed to fire both early and late discharges to a single stimulus. The sensitive dependence of latency and train length of response upon stimulus intensity was demonstrated. A tonal stimulus was found to suppress discharges of some spontaneously active units, while others discharged for varying lengths of time during presentation of a tone. Units sensitive to tones were as a rule optimally sensitive to restricted frequency bands; for the posterior segment of the

first auditory field most units observed were optimally sensitive to low frequencies, confirming in a general way the pattern of frequency distribution in auditory cortex previously established with different techniques. The repeated observation at several levels of the auditory system that single elements may be inhibited by some frequencies while excited by others appears to be one way in which central nervous action sharpens the pattern of activity evoked by tonal stimuli, providing, it is surmised, a mechanism for refining pitch discrimination.

Behavioral studies.—Butler, Diamond & Neff (54) find that after bilateral ablation of auditory areas I, II, & Ep, combined with removal of somatic area II, the cat's ability to discriminate changes in frequency remains essentially unimpaired. The contrast with the earlier findings of Meyer & Woolsey is explained by the fact that the latter authors included in their removals a good part of the "extra-auditory" temporal fields (188). In view of these findings these latter fields must be considered as auditory, and especially in view of the fact that more extensive degeneration in pars principalis of the medial geniculate is produced by their additional removal, than is by removal of AI, AII and Ep alone [Rose & Woolsey (227)]. Neff *et al.* (202) report also that ablation of AI, and in some cases AII and Ep as well, causes a severe deficit in the cat's ability to localize sound in space. A latter paper from the same group reports that cortical lesions including AI, AII and Ep produce a severe deficit when hearing is tested in a somewhat more subtle manner, by asking the cat to discriminate between sequences of tones which differed only in their temporal patterns (89).

Hood has published an informative study of auditory fatigue and adaptation in humans (144), and Dix has reviewed the subject of loudness recruitment in humans with disease or injury of the cochlear receptors (91). Many other papers dealing with hearing and vestibular sensibility in normal and diseased humans, as well as the entire field of psychoacoustics, are regarded as beyond the scope of this review.

THE VISUAL SYSTEM

Only those papers describing studies of the visual system which are of general significance for central nervous function will be described here, for a full scale review of the subject appears elsewhere in this volume. Following Granit's demonstration that stimulation of the reticular formation of the midbrain tegmentum produced long lasting augmentation of the spontaneous or light-driven activity of retinal ganglion cells, in the absence of antidromic activity in optic nerve fibers (122), Dodt has now found action potentials of efferent elements in rabbit retina which, with optimal placement of the stimulating electrode in the tegmentum, can be recorded in isolation from afferent elements (92).

The controversy over the fiber spectrum of the optic nerve continues. Chang now appears to have shown by measurements of conduction velocities that the action potentials of the optic nerve produced by stimulation of optic

tract are marked by three peaks, indicating groups of fibers conducting at modes of 70,30, and 17 m./sec. (63). The fibers of all three groups are found in the crossed projection, with differential spatial distribution in the contralateral optic tract, while only the first and third groups are detectable in the uncrossed projections. These observations conflict with those of P. O. Bishop *et al.* (26) and of G. H. Bishop & Clare (25) who recognized two and four groups respectively. Apparently the matter must be left unsettled.

Evarts & Hughes and Marshall show that tetanization of the optic nerve in pentobarbitalized cats for periods of 5 secs. to 6 min. produces a profound depression of the postsynaptic geniculate cell bodies, shown to last for hours (101, 102, 147). Such a long lasting change in excitability is a new observation. Recovery from this depression is produced by a second interpolated tetanic stimulation, the so-called post-tetanic potentiation, which may explain why the prolonged depression was not seen if the initial stimulation lasted 10 min. Neither the prolonged subnormality nor more than minimal signs of post-tetanic potentiation appeared in unanesthetized preparations (*cerveau isolé*). The fact that tetanic stimulation can increase the ratio of discharge to activated zone in synaptic relays depressed by some factor has been a common observation at other locations, and it remains to be seen whether post-tetanic potentiation is of any physiological significance in relay nuclei of afferent systems. That it is a normal event in sympathetic ganglionic transmission and in spinal cord reflex actions seems firmly established.

Visual cortex.—Bremer & Stoupel report carefully controlled studies of the polyspike response of visual cortex (32). The three initial deflections are unaffected by local strychnine or anesthetic, suggesting that all three are presynaptic in origin, yet only one can be recorded from the white matter after removal of cortex. Further, only latency and not interspike interval varies as stimulation site is changed from optic nerve to geniculate, and to radiations. These apparently conflicting observations fit the hypothesis presented by the authors, that the geniculostriate fibers divide upon entering the cortex into branches of quite different conduction velocities. The authors conclude, then, that the successive deflections of the cortical response are not signs of successive synaptic relays within the cortex, nor do they indicate a triple conducting system of thalamocortical fibers. This partially confirms but in part disagrees with earlier studies of the visual cortical evoked response by Chang & Kaada (64), Malis & Kruger (181), and by G. H. Bishop & Clare (24). In two other papers Bremer reports that the visual cortical response evoked by stimulation of an homologous contralateral cortical point, the callosal response, presents a polyspike form identical with that described for auditory system by the same author (30,31). That callosal volleys elicit such a polyphasic response is denied by Landau & Clare (164), and by Peacock (211). Bremer & Stoupel (33) show that this sequence is a general phenomenon of the primary receiving areas, when they are activated by synchronous thalamocortical volleys, an observation confirmed by Landau & Clare (164).

These controversies pertain to the problem of the neural mechanisms in color vision. Chang had previously suggested that "trichromatic vision" in the cat depends upon a triple conducting pathway from retina to cortex (62). Lennox now reports that light flashes of different spectral composition evoke cortical responses of different latencies and intensities (170). This occurs even when latencies to geniculate are equated, and when intensities are adjusted by comparing retinographic responses, all suggesting to Lennox that impulse transmission between geniculate and cortex differs according to the wave length of the stimulating light. These results are, however, difficult to interpret as evidence for color vision, in view of the clear and emphatic evidence from psychophysical studies that cats are unable to discriminate between colors (88, 133, 187). Lately Cohn, in conformity with these results, has been unable to find spectral sensitivities for single neurons of the geniculate or cortex (70).

PAIN

A reviewer of the year's literature is struck by the rarity of fundamental work on the neurophysiology of pain. As much as anything else this seems to be attributable to the emphasis upon the psychic qualities of man's reaction to pain, so much so that such phrases as "qualé of the soul" are considered to be adequate descriptions of this sensory experience (137). There is great reluctance to admit that an animal's experience is in any way comparable to that of man. Strictly speaking, of course, we do not know that an animal hears, or sees, or feels in a way comparable to the human experience of hearing, seeing or feeling, yet all would certainly agree that a considerable advance in knowledge of these afferent systems and of sensation, and perhaps of perception, has followed the intensive study of these systems in animals over the last two or three decades. It is in order to proceed with the study of pain in animals on the basis of two assumptions: (a) that a stimulus, such as radiant heat, which at a certain intensity evokes painful sensations in man, will evoke a similar sensory event in an animal, and (b) that knowledge of that event in the animal will bear directly upon the nature of the experience in man. This should lead to a detailed study of the events occurring in peripheral tissues subjected to painful stimuli, to the patterns of activity evoked in peripheral nerve fibers by it, the projection pathways and central destinations of that evoked activity, etc. The methods of physiological psychology should reveal some of the attributes of the response to pain in intact animals.

A prime example of the nonscientific point of view which prevails in some quarters is detailed in the review on the nature of pain by Goody (121). This paper begins with the premise that both the temporal and the spatial patterning of impulses in the nervous system are of some importance. Grasping firmly this everyday tool of the neurophysiologist the author is led, no longer earthbound, to the following statement, given in context and in its entirety: "A pain is always a *pain*, a quality like goodness, or justice.

Therefore *there can be no pathways nor nerve endings for pain*" (italics Goody's). Perhaps so, but the removal of pain from the field of busy scientific enquiry will most certainly delay any further understanding of it.

A symposium on the nature of pain has recently appeared (166). In it Haugen (140) summarizes some current concepts of the pain process, and Beecher (16) cogently and critically discusses the question of experimental pain in man, and its usefulness (or lack of it) as a method of assay of the analgesic action of drugs. He expresses doubt that there is any reliable correlation between the experimental pain threshold level and the suffering experienced by a patient. On the other hand, Hardy describes a uniformity of the pain threshold in man elicited by thermal radiation at a skin temperature of $45 \pm 1.7^\circ\text{C}$., the threshold for tissue damage (137). In another study Hardy *et al.* have made a quantitative assay of the effect of thermal radiation upon rat skin, and the animal's response to it (138). Skin twitch was evoked at 45 to 46°C ., and escape reactions at 51 to 52°C .

Harpman & Whitehead (139) have reinvestigated the phenomenon of cutaneous hyperalgesia and flare following local injury. The neural system responsible is the afferent group of nerve fibers subserving pain (if there is such a group!) [Goody (121)]; sympathetic fibers are not essential. The nature of the substance produced in damaged tissue eliciting these reactions is not known, so that it is not yet possible to integrate the roles of chemical and neural mechanisms. Armstrong *et al.* have isolated a pain-producing substance (PPS) present in blister fluid, inflammatory exudates, and in cell free plasma (10). PPS is not histamine or 5-hydroxytryptamine; it is probably a polypeptide similar to bradykinin or angiotonin.

The spinothalamic tract has been shown in monkey to have a heavy spinoreticular component, connections presumably playing a role in reticular activation of the forebrain [Rossi & Brodal (230)]. A comparative study indicates that there is a relative increase in the true thalamic component of this system from two per cent in marsupials to 20 per cent and 30 per cent respectively in monkey and chimpanzee. Whether this phylogenetic trend indicates an increasing dorsal thalamic, and hence cortical, projection of pain is uncertain, for there is no way of knowing which spinothalamic modes are responsible for this increase [Mehler (186)].

The question of whether pain sensibility projects to higher levels remains an enigma, although Silver describes a fascinating patient in whom a large cavernous angioma of the left parietal cortex produced intractable pain in the right arm, a symptom completely relieved by removal of the tumor (235a). Biemond argues, on the basis of patients with cortical and subcortical lesions showing diminution of pain sensibility over the contralateral body surface, that pain projects to the cortex, and makes the suggestion that it may project upon the second somatic area (22a)! Monnier presents a study of four patients in which coagulation of the ventral posterior nuclei of the thalamus relieved patients from severe and intractable pain of the contralateral side of the body (190). Some of the severe sequelae which limit the usefulness

of this procedure are discussed by him. The results add considerable strength to the idea that pain is projected upon the ventral posterior complex in the same topographic pattern as touch, and probably via the spinothalamic system.

MOTOR MECHANISMS

CORTICAL MECHANISMS CONTROLLING MOVEMENT

The persistent efforts of Woolsey and his colleagues during the past several years have resulted in a redefinition of the areas of the frontal lobes controlling movement. As a result one of the major controversies of the modern era of neurophysiology seems near solution, and the stage is set for study of the dynamic role of the cerebral cortex in the initiation and modulation of motor activity, studies which have in fact already begun. In Woolsey's experiments conditions were sought which would greatly reduce intrinsic cortical activity, by anesthetics, and thus reveal in isolation the background grain or pattern of efferent projections from precentral areas, evidenced by the movements elicited by threshold stimulating currents (261). A series of mammals have been studied ranging from rat to chimpanzee (255, 262). The most detailed reports deal with the macaque monkey. In this species the musculature of the contralateral half of the body (bilateral for lower parts of face) is represented in the precentral area as an image of the body form, head laterally, girdle, axial and proximal limb musculature anteriorly, tail medially, with the limb and distal appendicular muscles represented in an orderly way extending posteriorly into the depth of the Rolandic fissure. The precentral gyrus is differentiated in both dimensions. The pattern is distorted to allow a greater volume of cortex for the distal limb musculature involved in discrete and rapid movements. Threshold stimulation evokes very localized movements, but the authors emphasize the extensive overlap which exists, and which is made perfectly clear from the figurine drawings presented. This precentral motor area fits approximately area 4 of the macaque as defined by Brodmann, but includes not only areas 4a, 4b, & 4c of the Vogts, but also their area 6-a-alpha on the dorsal and lateral aspects of the hemisphere.

Although it has been known for a long time (145, 252) that stimulation anterior to the leg area of this precentral region can elicit arm movements, thus suggesting a second pattern, Penfield & Welch (213, 214) working with man and monkey and Woolsey *et al.* (261) with the monkey have now independently defined a supplementary motor area within which there is a complete reduplication of the representation of the bodily musculature. There is some evidence for a bilateral representation here. The supplementary area is located on the dorsal and medial surfaces of the hemisphere, anterior to the hip, tail, and leg portions of the precentral area, and extends medially onto the superior bank of the cingular sulcus. This area is apparently contained within area 6, but it does not fill it.

It should be emphasized that the precentral motor area and the supplementary area are not at all equivalent to and indeed are incompatible with the idea of a "motor" and a "premotor" cortex, as those concepts have been understood during the past two decades.

Redefinition of the motor areas requires restudy of the loss of function and the remaining motor capacities of animals after cortical lesions, made now in conformity with the new definitions. In two papers Travis (247, 248) describes such animals after removal of either of the two areas, and after various combined removals. Excision of the precentral motor area resulted in immediate severe impairment of voluntary motion, and hypotonia and diminished tendon reflexes in the contralateral extremities. No ipsilateral defects were observed. A partial recovery from paresis occurred within a matter of weeks, so that discrete movements were possible, though at about half normal speed. No spasticity developed. Defective, retarded, and hypermetric placing and hopping reactions reappeared after two or three weeks. On the other hand, lesions of the supplementary motor area produced "practically no paresis," but an enduring increased resistance to passive movements of the limbs, with hyperactive tendon jerks, most notable in flexor muscles. The spasticity demonstrated topographical localization according to the portions of the supplementary pattern removed. Grasp reflexes persisted for three to five weeks. Combined unilateral precentral and supplementary removals resulted in hypotonia, paresis, and impairment of reflexes lasting two to three weeks, which merged into a state of enduring spastic paralysis.

In another study Travis & Woolsey (249) have described not only the losses but also the remaining motor capacity of monkeys after bilateral removal of both the motor areas. This capacity is revealed best when massive ablations are made by seriatim partial removals, and with full scale use of massage, passive limb manipulation, and assisted functioning during the postoperative period. Such animals rapidly achieve ability to right, walk, and to use the hands in feeding, and in other activities involving prehension. Subsequent removal of the parietal lobes permanently abolished the use of the hands and produced an increased "ataxia," but the ability to right and to walk was recovered. These observations, combined with earlier ones which revealed that stimulation of parietal cortex still elicits contralateral movement after chronic ablation of the precentral motor fields (263), definitely establish that the postcentral somatic sensory area is also a "motor cortex." Two of the animals in the group of Travis & Woolsey survived complete ablation of all neocortex of both hemispheres, and subsequently retained the ability to right and to walk.

The retention of motor function after bilateral removal of the motor areas is emphasized also by the experiments reported by Pinto Hamuy (216). Such animals retain the ability to operate simple and complex problem boxes, but fail completely when the tasks require temporally organized movement sequences. While the retention of motor functions described is impressive, it is important to note that it is exactly those nimble movements

requiring speed and accuracy that are lost following removal of motor cortical areas, attributes of movement which are uniquely cortical in origin, as emphasized in the past by Marion Hines.

While the results of Woolsey and his colleagues show the pattern of the motor efferent projection—the least denominator of function—Lilly *et al.* have sought to bring out the full motor capacity of the cortex (172). In these experiments one hemicranium of monkeys was replaced with a molded plate bearing numbers of stimulating electrodes (in one case 613), and the animals were studied for survival periods of many months. Movements of one sort or another were obtained from almost the entire dorsolateral extent of the neocortex. The authors say:

These results suggest that motor functions are distributed throughout the anesthetized cerebral cortex in the normal state, and imply, when correlated with sensory maps, that each small area of cortex is truly 'sensori-motor' with a preponderance of one or another function.

Since the only overt result of any central nervous action is contraction of muscle or glandular secretion, Lilly's results mean that almost any group of cortical cells set into action may, via connections direct or complexly relayed, ultimately result in movement. There is no contradiction between these observations and those of Woolsey, which were obtained under quite different experimental conditions, and designed to answer different questions. The task now is to define more precisely the dynamic interrelations between cortical areas and afferent and efferent systems. Indeed, a few studies reported during the year point already in that direction.

Li finds that volleys originating in the nucleus ventralis lateralis exert an inhibitory effect upon neurons of the motor cortex (171). Creutzfeld *et al.* (78) report the results of a single unit analysis of motor and visual cortices, activated by direct electrical stimulation or by callosal volleys. Such forms of stimulation produce complex patterns of excitation and inhibition of neurons. What is needed first of all is an analysis of the action of single elements of the precentral motor area during movement in the normal waking animal. This will soon be possible by use of the developing methods of chronic implantation of microelectrodes.

Brookhart & Zanchetti have analyzed the variations in responsiveness of motor cortical cells by recording the discharges from them in fibers of the pyramidal tract (39). During the positive and early negative phases of cortical augmenting responses, evoked by repetitive stimulation of thalamic relay nuclei, the responsiveness of the efferent system is greatly increased, as it is also during spontaneous spindle activity, though it is completely unaffected by recruiting responses evoked by stimulation of intralaminar thalamic nuclei. Parma & Zanchetti indicate that increased responsiveness during augmentation is blocked by stimulation of the reticular activating system, though such stimulation does increase the background pyramidal activity (209).

Pyramidal tract.—The origins, course, and terminations of the pyramidal system continue to excite the interest of investigators, and a symposium has been held on this subject (167). Bertrand (21) traces efferent pathways from the supplementary motor area in the monkey directly to the pyramidal tract, with a much more important ipsilateral projection than from the precentral motor area, an observation which tends to confirm the bilateral nature of the supplementary system. With control of spurious excitation of afferents, Landau (162) maps the area of cortex activated by antidromic pyramidal volleys as coextensive with the areas of giant pyramidal cells in cat, a much more limited region than that mapped by previous investigators using the same technique. In the face of good evidence that the postcentral parietal homologue is a motor area, and indeed contributes 20 per cent of the pyramidal fibers, these results are difficult to understand. It may be that in Landau's experiments the entire pyramidal spectrum was not excited, but only its large fiber fraction. Chambers & Liu (61) redefine the areas of origin of the pyramidal tract in the cat and show a lack of localization throughout its course as Barnard & Woolsey have shown in rat and monkey (13), but by using stains for degenerating terminals they show also a very precise localization in spinal cord segments of the endings of pyramidal fibers.

Walshe has reviewed the entire subject of the significance of the Babinski phenomenon in relation to pyramidal tract lesions in a manner at once amusing and instructive (254). He makes very clear the fact that it is part and parcel of the nociceptive flexion reflex in man.

THE EXTRAPYRAMIDAL SYSTEMS: ABNORMAL MOVEMENTS

Segundo & Machne (233) have attempted to define sources of input for the subcortical gray masses of the basal ganglia. Using the method of single unit analysis in unanesthetized cats, they show that the responsive two-thirds of units observed in the putamen and globus pallidus respond to a variety of somatic afferent stimuli, while cells of the former may also be inhibited by such stimuli. Infrequently, the same single units responded to both somatic and vestibular or vagal afferent excitation. Cells of the claustrum displayed more marked modality convergences, between somato-olfactory and somato-visceral inputs. The lack of topographical pattern, the long and variable latencies, and the extensive modality convergences set this system apart from a specific afferent projection system, and its physiological significance is unknown. This activation may be a part of the general arousal reactions, and the afferent pathways to basal ganglia may even tranverse the cortex itself.

Forman & Ward (108) have observed the motor responses of waking, freely moving cats stimulated via electrodes chronically implanted in caudate nucleus. Stereotyped movements of the contralateral bodily musculature were elicited, which were present both before and after chronic ablation of the motor cortex; a somatotopic localization was demonstrated within the

head of the caudate nucleus. Inhibitory phenomena were not observed under any conditions of these experiments.

Tremor has been shown to be a normal phenomenon in healthy subjects, occurring at frequencies between 5 and 15 per sec. Amplitude but not frequency is affected by load. This is discussed from the standpoint of "servo-instability" in stretch reflex arcs (111, 134), and from the cybernetic approach (238). Stark & Sherman (239) have made a servoanalytic study of the consensual pupillary reflex to light, considering it to be an error-actuated servosystem of low gain and great stability.

The major impetus towards a better understanding of the function of the extrapyramidal system comes from those clinicians caring for patients with tremor and spasticity. While the subject is still exceedingly controversial (224), there can be little doubt that surgical destruction of the pallidum and ansa lenticularis provides a new and potent means of treating this disabling condition. What catastrophes the treatment itself may bring are yet uncertain. Of the many excellent descriptions of the procedures employed and the results obtained, attention is directed to the book of Cooper (76). These clinical observations as well as those of Forman & Ward cast doubt upon the generally accepted notion that the basal ganglia are major sources of descending inhibition impinging via multiple relays upon the segmental motor apparatus. Abnormal movements and rigidity are apparently associated with temporally altered patterns of discharge from the basal ganglia, the removal or interruption of which alleviates these symptoms and signs.

Several papers have appeared which report studies of the extrapyramidal tracts of the brain stem, and their ascending and descending connections. Bebin describes the composition of the central tegmental tract of Becterev in the monkey as a complex bundle of fibers of varying length interconnecting the mesencephalic periaqueductal grey, tegmentum, red nucleus, and the inferior olive (15). Isolated lesions of this tract produce palatal nystagmus, an association originally observed in man by Foix. Verhaart has described the same periaqueductal gray-rubro-olivary course of this tract in man (250).

Walberg describes a multitude of descending connections which terminate in the inferior olive, from the sensorimotor cortex directly, from the caudate and putamen, and most heavily from the periaqueductal grey and the red nucleus (253). These latter make up a large part of the central tegmental tract studies by Bebin and Verhaart. Torvik (244) details the transneuronal changes of inferior olive and pontine nuclei after lesions of systems projecting upon them. Scheibel *et al.* (234) have studied in Golgi preparations the types of synaptic terminals of afferents to the olive, and olivary cell types as well, and correlated the latter with the olivary efferent projections.

Orioli & Mettler (204) show that section of the rubrospinal tract in monkey produces ipsilateral hypokinesia, and tilting of the head and body towards the side of the lesion, but no other permanent deficiencies. Carpenter (55) finds similarly that an enduring hypokinesia and body rotation or tilt

follow lesions of the red nucleus itself; lesions of this region produce cerebellar signs only if both ascending and descending limbs of the brachium conjunctivum are destroyed [Orioli & Mettler (206)]. Section of the olivary decussation also produces hypokinesia and no other signs, and even this may be due to associated damage to vestibular structures (205).

BRAIN STEM INFLUENCES UPON SEGMENTAL REFLEX ACTIONS

Several papers have appeared which describe in some detail the action of reticular and vestibular descending systems upon spinal cord reflex actions. Brooks, Koizumi & Siebens (40) report that stimulation of inhibitory reticular areas usually produced a generalized inhibition of both flexor and extensor monosynaptic reflexes; occasionally a reciprocal effect was obtained. The inhibition commonly outlasted the cessation of stimulation by 2 to 3 min. Several properties of this inhibition were determined: it facilitates antidromic invasion of the motoneurons, does not prevent or terminate orthodromic post-tetanic potentiation, or interfere with orthodromic facilitation of antidromic invasion. These data lead the authors to suggest that this inhibition does not block afferent fiber terminals, but acts postsynaptically, perhaps by hyperpolarization of the motoneuron soma, with the proviso that such hyperpolarization cannot be of such degree or location as to interfere with antidromic transmission over the region of the axon hillock. Hugelin & Bonvallet (146) describe somewhat similar effects of reticular stimulation upon the monosynaptic pathway from trigeminal afferents to the masseter muscle nerve. Koella *et al.* (155) have determined by an ingenious method the quantitative interrelation between the degree of imposed muscle stretch, position of the head in space, and the resulting muscle tension. The tension increment produced by a particular stretch increases as rotation proceeds from prone to supine positions around the longitudinal axis. The greater the initially imposed stretch the greater will be the vestibular influence; stretch afferents and vestibular receptors exert their influence upon motoneuron excitability in a quantitatively interdependent fashion.

Employing single fiber recording from thin ventral root filaments Andersson & Gernandt (7) have studied directly this interplay of vestibular and stretch afferents upon the activity of alpha and gamma motoneurons. Their results indicate that the gamma efferents are initially excited by weak vestibular afferent stimulation, but that stronger vestibular input activates both groups. From their study of the combined vestibular and stretch afferent play upon motoneurons they emphasize the powerful autogenetic control of motoneuron activity by tendon organ afferents, which can completely dominate even the most intense vestibular excitatory influences.

REFLEX ACTIVITY OF THE SPINAL CORD

Synaptic actions on motoneurons.—The year has been marked by the publication of Eccles' book *The Physiology of Nerve Cells* (96), which summarizes a wealth of experimental data on excitation and inhibition of

motoneurons. Combining methods of intracellular recording of potential changes and iontophoretic manipulation of the cell's internal ionic composition, the author and his colleagues show that the ionic hypothesis of excitation and conduction in nerve fibers developed so successfully by Hodgkin & Huxley applies with equal validity to nerve cells. The mechanism of inhibition is similar in principle but opposite in direction, depending upon permeability changes to K^+ and Cl^- , but not to Na^+ , produced by a specific transmitter yet unknown. Beyond detailed experimental results, this important monograph develops several ideas of general significance: (a) specific nerve cells are likely to be specific in their synaptic action upon other nerve cells, either excitatory or inhibitory but not both; (e) a given nerve cell releases at all its endings only one transmitter agent (Dale's principle); (c) single neurons achieve integrative action by virtue of the fact that excitation occurs in a local region of lowest threshold, the "initial segment" or axon hillock, which is continually conditioned by subliminal membrane phenomena occurring in dendrites, and perhaps in other regions of the soma, which may be either excitatory (depolarizing) or inhibitory (stabilization of membrane potential); (d) plasticity of form and function is a likely property of the central nervous system, and deserves intensive study.

The time constant of the motoneuron membrane has been measured at 1.0 msec. [Frank & Fuortes (109)], and at 2.5 msec. [Coombs, Curtis & Eccles (74)]. Though different, both values are too short to account for the prolonged time course of the local excitatory postsynaptic potential as a passive restitution of membrane charge, and indicate that a residuum of excitatory transmitter persists during the decaying phase of the postsynaptic potential. Fatt (103, 104) has studied the sequence of events during both synaptic and antidromic activation by intracellular recording, and by mapping the extracellular potential gradients about single active motoneurons. The two-phased development of the motoneuron action potential evoked in either manner, with an inflection point at about 30 mv. depolarization, is interpreted by Fatt to represent sequential activation of cell body and dendrites. Centrifugal conduction in the latter was determined to be at a rate of 0.7 to 1.0 m per sec. For Eccles (96), and for Fuortes, Frank & Becker (114), these two phases indicate activation first of the axon hillock and then of the cell body proper. Regardless of those differences the important point is made by all that the axon hillock is a region of low threshold at which cellular activation takes place, with the likely possibility that centrifugal conduction then occurs towards other parts of the neuron. This marks a revolution in concepts of the sequence of events in intercellular transfer and postsynaptic discharge. Whether dendrites ever conduct action potentials, and if so in which direction, seems to be a matter of considerable debate; that synaptic action upon them will condition cellular excitability is clear.

Rhythmical activity in reflex pathways.—Attention turns from analysis of the physiological properties of reflex pathways activated by single synchronous volleys to observations of their behavior under steady states of re-

petitive drive. Significantly for this trend, Granit, Henatsch & Steg (124) have shown that among alpha motoneurons previously thought to be homogeneous two types exist: (a) those which discharge repetitively for long periods of time under steady stretch drive, and (b) those which respond only at onset of maintained afferent input. Tonic motoneurons are thought to be smaller than phasic ones, and their fibers make up the lower register of the alpha motoneuron fiber spectrum.

In a detailed and extensive investigation Lloyd & Wilson (175, 176, 177) have studied the properties of the monosynaptic reflex pathway when repetitively activated at various frequencies. At low frequencies a severe reflex depression occurs, which originates by action of the I-a afferent fibers responsible for monosynaptic reflex transmission. The depression is present at frequencies as low as 6 per min., and observation disturbing to investigators accustomed to repetition rates of 1 per sec to 0.3 per sec in central nervous system studies! At frequencies between 60 and 100 per sec. temporal facilitation occurs. The time course of the temporal facilitation is described by an exponential function falling to $1/e$ in 4 msec., and is thus attributable to the well known postsynaptic potential-residual facilitation, and to no new property. Motoneuron subnormality limits the upper rate of rhythmic firing. These properties of the pathway were determined by both a population and an elegant single unit analysis of the pools of neurons under study. For Lloyd the observations of the reflex pathway under repetitive drive are explicable in terms of the properties already known, while Fuortes (112) suggests that during rhythmical activity an excitatory event grows by temporal summation and is transformed into an oscillating event, a property qualitatively different from those features of transmission determined by study of reflexes evoked by isolated volleys. The enduring depression observed at low frequencies by Lloyd has been studied also by Beswick & Evanson, who have reached similar conclusions (22). These authors observed also that the direct inhibitory effects of group I volleys show no changes during repetitive stimulation at a low rate.

Fuortes & Hubel have compared flexor and extensor reflexes of muscular origin in decerebrate cats, finding that extensor motoneurons fire more easily at high than at low frequencies, while flexor motoneurons respond to each volley at low frequencies, but to only the first of a series of afferent volleys delivered at high frequencies (113). The authors suggest that in the decerebrate state a property favoring temporal summation is present in the extensor but not in the flexor pathways.

The hypothesis that circulating activity in re-entrant interneuronal circuits is responsible for flexor reflex after-discharge, originally proposed by Forbes, has been tested by Burns (46). The after-discharge of frog's flexor reflex was always terminated by d-c current stimuli delivered across the entire cord. On the assumption that such a stimulus simultaneously depolarized all participating interneurons with subsequent simultaneous recovery cycles and cessation of circulating activity, the hypothesis is sup-

ported. Wilson finds post-tetanic potentiation to be a property of the polysynaptic reflex pathway through the cat's spinal cord (257); the phenomenon is well established for the monosynaptic pathway.

Patterns of reflex action.—The first description of crossed reflex activity analyzed by modern techniques has been given by Perl (215). When afferent volleys in cutaneous nerves are confined to the beta-gamma group (14 to 6μ), both contralateral and ipsilateral flexor motoneurons are facilitated, a form of bilateral simultaneous innervation. Signs of the classical crossed extensor reflex were evoked when afferent volleys included the delta pile (6 to 2μ) of cutaneous afferents. Eccles, Eccles & Lundberg have determined the pattern of group I-a interconnections between muscles (97). Muscles with closely interrelated functions, i.e., synergists, exhibit a high degree of monosynaptic linkages, but some overlapping between muscles belonging formally to different myotatic units was found, and some relations are unidirectional only. Motoneurons of red slow muscles have a wider group I-a receptive field than do those of pale fast muscles.

By means of intracellular recording the I-a and I-b components of the large afferent fibers from muscle have been correlated with their central synaptic effects upon spinal motoneurons, conforming is full to the myotatic-inverse myotatic reflex pattern [Eccles, Eccles & Lundberg (98).] Laporte & Bessou have confirmed this relation of the I-a afferents with annulospiral spindle receptors and of the I-b group with Golgi tendon organs (165). Plotting of the potential fields produced within the spinal cord by such volleys indicates that Group I-a fibers relay in the intermediate nucleus of Cajal. Groups I-b and II have more extensive terminal distributions, but they are concentrated in the intermediate region of the spinal gray, while group III muscle afferents relay in the dorsal horn, dorsolateral to the intermediate nucleus [Coombs, Curtis & Landgren (75)]. Using a similar method, Molina & Gray have plotted the activity in spinal gray matter evoked by volleys in cutaneous nerves. All primary afferents terminate in the dorsal horn, which contains also the second order neurons of the cutaneous afferent pathway (189).

Matthews & Rushworth find that local procaine selectively blocks gamma before alpha motor fibers (184, 185). Using this method in a study of the phasic and tonic aspects of the stretch reflex, they find that maintained reflex discharge to steadily applied stretch depends upon the activity of gamma efferents; resting tone was eliminated by selective block of gamma fibers, without affecting phasic responses to tendon jerks.

Neuropharmacology of spinal reflex pathways.—The great amount of detailed information about the intrinsic mechanisms of the stretch reflex pathway makes it an ideal system to use in studies of the action of drugs or other extraneous substances upon the central nervous system. Drawing upon an extensive background of techniques and knowledge, V. B. Brooks, Curtis & Eccles have studied the effect of tetanus toxin upon motoneurons (41). They show very clearly that this substance exerts its central effect by blocking

synaptic junctions which are inhibitory in nature. The removal of all inhibitory influences is presumed to account for the unrestrained motor activity characteristic of poisoning by tetanus toxin, for no direct excitatory action was observed. In a somewhat similar study, Curtis & Eccles have observed the central action of nicotine and acetylcholine on spinal motoneurons given by rapid close arterial injection (80). Both substances produce a transient depression of monosynaptic and polysynaptic reflexes, both flexor and extensor, by a transient excitation of the Renshaw cells, which exert a powerful inhibitory action upon motoneurons. Epinephrine, norepinephrine, succinylcholine, methylcholine, carbaminocholine, and ATP delivered intra-arterially had little or no immediate action on spinal reflexes, but the first two had a potentiating effect after a long delay (8 to 10 secs.), an effect observed also by Wilson (258). This latter author has not observed the reciprocal action of epinephrine on extensor and flexor reflexes previously described by others.

THE CEREBELLUM

The work of recent years has further confirmed the fact that the cerebellum is a highly organized center which serves to regulate muscular activity. Informed of steady states and transient change by all the major afferent systems (olfactory yet to be shown) it is reciprocally connected by multisynaptic pathways with wide regions of the brain stem, the extrapyramidal grays and the cerebral cortex, in a highly organized fashion, and is thus capable of "modifying muscular action from the level of its inception to that of its execution" (141). The work of the present year is in the main concerned with the further study of these connections, and of their functional meaning.

Afferent connections.—Combs (72, 73) has found, by means of electrical stimulation of brain stem and cerebellocortical recording, and by nerve volley-evoked potential recording before and after various brain stem lesions, that the lateral reticular nucleus is the essential relay for the localized projection found in the anterior lobe of the cerebellum. The nonlocalized projection system may relay here, as well as through external cuneate- and olivocerebellar systems; neither of the last two are essential for the localized projections. Morin and his colleagues confirm the somatotopic pattern of projection to paramedian lobule, although such a pattern is lacking in the ascending spinal systems responsible for it (161, 194). Goldman & Snider describe two relay pathways through the cerebellum, one involving only one synapse through central nuclei into efferent projections, and a second traversing the cortex, involving two or more synapses (120).

Intrinsic mechanisms of cerebellar cortex.—Granit & Phillips (126, 127) have applied the method of single unit analysis in a study of the excitatory and inhibitory processes acting upon Purkinje cells driven by stimulation of either cerebellar afferents or of the cortex. Slow spontaneous rates of Purkinje cell discharge are greatly increased by tetanization of cerebellar nuclei or

nucleocortical fibers, excitation outlasting the period of stimulation. Discharge of a cell follows development of a gradable prepotential, in classical fashion. Inhibition is produced by either membrane hyperpolarization or by excessive depolarization (their "inactivation process").

Efferent systems.—In three papers Moruzzi & Pompeiano (197, 198, 199) present evidence that classical cerebellar inhibition of ipsilateral decerebrate rigidity is mediated via rostralateral fastigial neurons. After lesions of this region, cerebellar vermal stimulation accentuates decerebrate rigidity via an ipsilateral facilitatory system which relays through rostromedial fastigial neurons. These two antagonistic systems arise from lobules III, IV, and V of the vermis; the augmenting influence is normally concealed by the dominating inhibitory response to cortical stimulation, but may contribute to postinhibitory rebound. This differential effect of cortical stimulation upon rostrofastigial neurons has been confirmed in a single unit analysis by Arduini & Pompeiano (9). Some 70 per cent of rostralateral neurons are facilitated and an equal share of rostromedial elements inhibited by such stimulation. The cortical origins of these two systems are thought by the Italian workers to overlap widely, but the earlier work of Hampson, Harrison & Woolsey indicates that they cannot be completely coextensive (135). The latter group found that ipsilateral extensor tonus was inhibited by stimulation of the medial strip of the anterior lobe vermis, while stimulation of the intermediate zone produced ipsilateral extensor facilitation.

A third fastigial efferent system is shown by the fact that lesions limited to the caudal portions of the nucleus result in a striking decrease in contralateral extensor tonus. That this release is a dynamic asymmetry is shown by two facts: (a) it is restored by section of the dorsal roots innervating the paired ipsilateral limb, and (b) bilateral extensor tonus is restored by an additional lesion of the caudal portion of the second fastigial nucleus. In a fourth paper Batini, Moruzzi & Pompeiano have reported a study of the alpha rigidity of the limbs which persists in decerebrate cats after interruption of the myotatic loop by dorsal root section [(14), see also (125)]. This alpha rigidity is normally concealed by both descending cerebellar and ascending Schiff-Sherrington (for the forelegs) inhibitory influences.

Single folia of lobules IV or V of the vermis project in a strictly localized way upon medial reticular elements, thus revealing a somatotopic patterning in the cerebello-reticulospinal system (117). That this efferent system projects also upon Deiter's vestibular nucleus is shown by the single unit analysis of DeVito, Brusa & Arduini (87).

By staining degenerating terminals after fastigial lesions, Thomas *et al.* have shown a heavy ipsilateral fastigiobulbar projection upon all vestibular nuclei, a lesser one to the reticular formation, a heavy contralateral projection to the reticular formation, but a weaker one to vestibular nuclei (242). An ipsilateral ascending fastigial projection via the brachium conjunctivum described by these authors is denied by Carpenter & Stevens (57) who, using the Marchi method, have detailed the ascending projections of the dentate and interposed nuclei through the brachium conjunctivum.

Cerebellocerebral projections.—Ruwaldt & Snider report that stimulation of the vestibular areas of the cerebellum, the flocculonodular lobe, uvula and lingula, evokes electrical responses in middle and anterior ectosylvian and anterior suprasylvian gyri of the cerebral cortex (232). This projection area completely encompasses the cortical projection area of vestibular receptors, previously described by several investigators. This finding further supports the generality that afferent projection areas of cerebral and cerebellar cortices are reciprocally interconnected.

REGULATION OF ACTIVITY LEVELS IN THE CENTRAL NERVOUS SYSTEM

THE RETICULAR FORMATION

The work of the last decade, summarized in several recent reviews (28, 47, 83, 196), has shown in general outline that three systems exist in that region of the brain stem somewhat vaguely designated the reticular formation. The first, activated by all afferent systems as well as by corticofugal ones, projects upwards upon the forebrain, maintains it at a level of activity called wakefulness; decrease in its drive lowers forebrain activity through a continuous spectrum, at one point called sleep, at another coma. Two other systems project downwards upon the segmental motor apparatus, the one facilitating and the other inhibiting reflex and voluntary movement; their normal balance results in normal tone. These two receive cerebellar, brain stem, and extrapyramidal input, funneling nonpyramidal outflow upon final common paths. The work of the present year is marked by efforts to further define these systems, and their functional meaning.

The avalanche of investigations of these systems leaves some confusion because of the frequent lack of clear descriptions of the regions in which stimulations, lesions, or recordings are made. More precise correlations between structure and function are highly desirable. The studies of Olszewski (203) have shown that the region is a much more highly differentiated one than had originally been thought, and is made up of many specific nuclei of different cell types which, it may be supposed, have different connections.

Sources of reticular drive.—Are reticular cells capable of an autochthonous activity independent of all input from sensory systems? Bonvallet, Hugelin & Dell indicate that to a certain extent this is indeed the case, for neurons of the mesencephalic reticular formation in a wedge isolated by mammillary and postcollicular sections are spontaneously active (27). Their rate of discharge is modified by changes in blood carbon dioxide and epinephrine. Earlier observations of the same group (84) showed that epinephrine induces cortical EEG arousal even when the mesencephalic reticular formation is deprived of sensory input by pontomesencephalic transection, a finding now confirmed and extended by Rothballer (231).

Nevertheless, the principal source of drive maintaining the waking state does come from afferent inflow. It is important to know the sources and the patterns of that inflow. Apparently it is not universally distributed, for in the unanesthetized animal only 50 per cent of the pontile and 35 per cent of

the midbrain reticular cells are driven by sensory stimuli or by impulses from the cerebellar or cerebral cortices [Palestini, Rossi & Zanchetti (208), Mancía, Melchese & Mollica (182)]. Contrary to earlier statements, convergence upon those which can be driven is far from universal. Cells in the medial two-thirds of the pontile region, the site of origin of long ascending axons [Brodal & Rossi (36)], are activated from several sources, but convergence upon those of the lateral reticular region or of the midbrain regions was exceedingly rare (182, 208). Obviously what is needed is a precise description of these projection patterns and their correlation with the detailed morphology of the region. The various sensory systems are not of equal potency in maintaining wakefulness, for Roger, Rossi & Zirondoli (225) find that section of cranial nerves I, II or VII separately, or all together, does not modify the vigilant state of *encéphale isolé* cats, while a section of the trigeminals alone results in a continuing somnolence, from which the animal can, however, still be aroused by sound or smell.

Recent anatomical and physiological findings indicate that an efferent system of widespread cerebral cortical origin projects upon the reticular area (229, 110), though the topographical details of this projection are not clear. Adey, Segundo & Livingston (1) have now assayed its functional significance: stimulation of the cortex conditions transmission through two conducting pathways ascending the brain stem, the one rapid and the other slow. The effects include blocking interaction or augmentation, or both in sequence. The most pronounced and enduring effects were induced by stimulation of the hippocampus, which recalls Nauta's description of the projection of the fornix system upon the central mesencephalic grey (201).

Mechanism of action of the ascending system.—How does the ascending system exert such profound effects upon the cerebral cortex? Dondey & Machne (93) make the comforting observation that EEG desynchronization produced by reticular stimulation is indeed an activation; during it single cortical neurons fire at a greatly accelerated pace, while during somnolence they discharge in short runs associated with spindles. Arduini, Mancía, & Melchese (8) show that reticularly provoked EEG arousal is accompanied by a negative d.c. shift of cortical potential of 200 to 400 μ V, which outlasts stimulation by 1 to 2 sec. and which is accentuated by strychnine locally applied to the cortex. The significance of such a slow potential change evoked by cortical afferents is uncertain [see also Dondey & Snider (94)], but one might infer that it represents maintained partial depolarization of dendrites, which have no refractory period and are capable of graded and summable activity (68). Primary cortical potentials evoked by single stimuli to thalamic relay nuclei are unaffected by electrocortical arousal provoked by reticular stimulation, but the augmenting responses associated with iterative stimulation of the same relay nuclei are reduced. Primary evoked potentials elicited by single peripheral nerve volleys are, on the other hand, greatly reduced by cortical arousal, due perhaps to the descending reticular control of sensory relay stations [Gauthier, Parma & Zanchetti (118)]. Beller, Gestring & Purpura

(19) suggest that corticobulbar systems and sensory input both contribute to "reticular awakening" which can, however, proceed in the absence of one of these sources of input. They find that ablation of auditory or somatic sensory cortex does not block generalized activation produced by auditory or sciatic nerve stimulation. It is difficult to fit these observations with the earlier one of Bremer & Terzuolo that, in the *encéphale isolé* cat, removal of the auditory cortex eliminates arousal induced by sound stimuli (35).

Purpura suggests an additional neurohumoral influence of reticular regions upon the cerebral cortex (219). When reticular stimulation is delivered to one of a pair of cross-circulated *encéphale isolé* cats electrocortical arousal occurs in the second animal after a delay of 30 to 80 sec. Controls apparently indicated that the effect did not result from the release of endogenous epinephrine into the circulation of the stimulated animal. The humoral agent postulated has not been identified. Obviously this work has opened what should be a fruitful line of investigation.

THE "DIFFUSE" THALAMIC PROJECTION SYSTEM

It is now fifteen years since the discovery by Morison & Dempsey (85, 195) that localized stimulation of certain thalamic nuclei, the intralaminar group, ventralis anterior, and reticular complex, evokes electrical responses of the cortex of long latency and widespread distribution, responses which gradually increase in amplitude during iterative stimulation at 6 to 8/sec. They constitute the "recruiting" response. In the intervening years a great deal of information has become available concerning the details of this system, thanks in large part to the important series of papers by Jasper and his colleagues, but the functional significance of this system is as difficult to assess at the present time as when first described [see (148)]. Buser has provided a thorough review of the subject, together with some stimulating hypotheses concerning the role of the system in cerebral function (47).

The nature of the thalamic structural substrate involved and its pathway to the cortex remains a challenging and fundamental aspect of the problem. Hanbery & Jasper (136) have shown sometime ago that recruiting activity is projected forward from the intralaminar group to the nucleus ventralis anterior and the oral pole of the reticular nuclear complex. The widespread cortical connections of the latter are invoked to account for the very widespread cortical pattern of recruiting responses. It should be pointed out that this is only possible if the assumption is made that there is universal activation within the reticular complex from any one of its parts, for each part has a specific and localized cortical projection; there is nothing "diffuse" or "nonspecific" about it (226). Powell & Cowan (218) now report that the intralaminar group projects in a specific and topographically organized pattern upon the basal ganglia (on this opinions differ). Although recruiting responses have been recorded in the neostriatum, and cortical recruitment can be evoked by stimulation of this structure, it is apparently not an essential link in the recruitment pathway (3). Does this suggest that the re-

cruitment produced by stimulation of intralaminar nuclei is the result of activation of the fibers of passage with which they are so heavily invested?

Kerr & O'Leary have confirmed the general nature of the recruitment system in the rabbit, emphasizing the importance of the midline thalamic nuclei in provoking this phenomena (152). Some implications of the function of the system come from the report of Parma & Zanchetti that a partial occlusion occurs between the negative components of the recruiting and augmenting responses simultaneously evoked, and that the negative phase of the recruiting wave facilitates the positive component of the augmenting response (evoked by repetitive stimulation of the ventral-posterior thalamic nucleus) and the pyramidal discharge evoked by the latter (210). Jung *et al.* report that some single neurons of the visual cortex can be activated by stimulation of either the dorsolateral geniculate body or "nonspecific" nuclei, and that complex interactions between the two involving both facilitation and inhibition occur (150). These two reports indicate that specific sensory afferents and those of the recruiting system debouch at some stage upon the same cortical neurons, and imply a physiological regulating function for the latter.

Clare & Bishop present evidence that the recruiting response results from activation of cortical dendrites by an intralaminar-cortical system conducting impulses at C-fiber velocity, and that this response shows two periods of supernormality (69). The second occurs 1/6 to 1/10 sec. after initial excitation. Repetition of the stimulus, i.e., the intralaminar thalamocortical volley, at this rate causes an increase of the response during the second supernormal phase.

No reasonable hypothesis of the relation between the ascending reticular activating system and the thalamic recruiting system has yet found adequate experimental support.

INTRINSIC CORTICAL MECHANISMS

Bishop's review sets in perspective a most important new development in current thinking about the functions of nervous systems, and in particular of the cerebral cortex. That is, that the all-or-none explosive conduction characteristic of nerve fibers is an exceptional, and by no means the predominant, form of nervous activity (23). Bishop's thesis is "that the chief and the most characteristic functions of nervous and other excitable tissues are performed by means of graded responses." Neural actions can not be regarded as digital functions. Further,

If various excitable cells or parts of cells (e.g., cell bodies and their dendrites) are to be compared on the basis of some fundamental process which they are all supposed to exhibit, the special case of the all-or-none propagated impulse is probably the least likely to furnish useful criteria for the comparison. Nothing is more obvious in this connection than that the functioning neuron, however it is excited by all-or-none impulses from axons, or transmits all-or-none impulses to its own terminal processes, has among its essential functions properties that are not merely all-or-none, but more-or-less (68).

It has been known since the work of Adrian (2) that the dendrites of cortical pyramids are capable of responses lasting 10 to 15 times longer than the impulses in axons of those same cells. Largely due to the work of Clare & Bishop it is now clear that the dendritic response can be graded, summed, and is capable of being prolonged to a more or less steady state, under steady drive (67, 68). It is suggested as a reasonable mechanism of the prolonged oscillating central states of excitation and inhibition postulated long ago by Sherrington. No one can doubt that an exciting and promising new field of cortical physiology has been opened by these investigations.

Clare & Bishop describe corticocortical pathways ending only upon the dendrites of pyramidal cells (67, 68). Activated by these pathways, the dendritic membrane displays a response of long duration (about 15 msec.), has no refractory period, supports local activity without propagation of impulses into the cell body, and thus alters by electrotonic extension to the cell bodies the excitability of the latter to impulses reaching them directly over axosomatic synapses. When the dendrites do conduct their conduction is decremental, occurs more readily away from than towards the cell body, and has a value of a small fraction of a meter per second (but see below the contrary opinion of Purpura & Grundfest). Following excitation dendrites are first supernormal for 20 msec., then depressed for 100 msec; the depression is associated with hyperpolarization. The absence of refractoriness permits repetitive stimulation to maintain a continuous negativity. Thus modulation of input to dendrites alone could induce potential wave forms of any duration, and the activity of dendrites appears to be such as appropriately to account for the potentials of the EEG. The 100 msec. period of depression may account for the alpha rhythm.

In a later paper Clare & Bishop (69) describe two types of potential-wave responses of apical dendrites of the cortex, evoked by intralaminar thalamic stimuli. A shorter latency one decrements when its afferents are stimulated at 6 per sec., or faster; a longer latency response increments at that rate, and accounts for the classical recruiting response of Morison & Dempsey (see section above). The implication is that the incrementing and decrementing dendritic responses are initiated at different sets of synapses on the dendrites.

Important contributions to the knowledge of the properties of cortical dendrites, and of central synaptic transmission, have been made by Grundfest & Purpura, and described in a series of papers, reports, and reviews (130, 131, 132, 221, 222, 223). For these authors the dendritic response is local and is not conducted, even decrementally, is generated always by transsynaptic activation: it is a true postsynaptic potential. The dendrites are electrically inexcitable. The subsynaptic electrogenic areas of dendrites are capable of only local, graded, nonconducted responses, are spatially juxtaposed to cell body membranes capable of explosive all-or-none electrogenesis. No morphological sign of demarkation between these two types of membrane at cell body-dendritic junction has yet been found, but if the change is a gradient, perhaps one would not be expected. The question arises how a local

response on apical dendrites, which may be some millimeters in length, could affect the cell body at all if limited to electrotonic extension, for the space constant of such narrow elements must be very small. A major contribution of Grundfest and his colleagues is the very clear demonstration that some afferents produce inhibitory transsynaptic action upon cortical dendrites, thus adding a second dimension to the modulating influence of the latter upon pyramidal cell bodies. Purpura (220), and Purpura, Houspian & Grundfest (222) report that the flattening of the electrocorticogram evoked by reticular stimulation (the "arousal reaction") is associated with and is possibly the result of a prolonged inhibition of the cortical dendrites. Using this knowledge of the excitatory and inhibitory synaptic influences acting on dendrites, Purpura & Grundfest have made an extensive study of their pharmacological properties. These investigations are reviewed in another chapter of this volume.

FURTHER ASPECTS OF ELECTROCORTICAL ACTIVITY

The associative and elaborative functions of the cerebral cortex commonly designated as "higher" and usually approached in the setting of experimental psychology are reviewed elsewhere in this volume. It will be seen there that significant advances have resulted from the combination of conditioning and electrical recording techniques. In addition, several recent papers point to the possibility of studying this "higher" or associative activity of the cerebral cortex by conventional electrical methods. Particular reference is made to the work of Buser & Borenstein, and to that of D. Albe-Fessard, reported in a series of papers and recently reviewed by Buser (47).

Visual stimuli evoke responses in the suprasylvian gyrus of chloralosed, unanesthetized-curarized, and waking cats with chronically implanted electrodes (48, 49, 53). These responses are half again as long in latency as those evoked at the same time in the primary projection area. They are found in two very restricted areas of the association cortex of the suprasylvian gyrus (study of the topographic pattern is not yet complete). Thus for several reasons they are not to be confused with the secondary responses of Forbes. The suprasylvian areas are also activated by auditory or somesthetic stimuli (5) in patterns which overlap somewhat, and interaction between sensory modalities has been observed, as Amassian had reported earlier (6). In the case of the visual system the afferent pathway is thought to involve intrathalamic relay from the dorsolateral geniculate body to the pulvinar; in that of the somesthetic system, from the ventral posterior nuclei to the centremedian nucleus, and thence to the cortex. In this regard Powell & Cowan's observation (218) that this nucleus projects to the neostriatum and not to the cortex should be considered.

Modulation of the association area responses by strychninization or local anesthesia of the related primary projection area indicates also a cortico-cortical input via the latter, but the association responses persist after removal of the primary receiving areas. The association area responses dis-

appear during light sleep, and are completely abolished during the "arousal reaction" provoked by stimulation of the mesencephalic reticular formation. While these responses are greatly exaggerated by chloralose, they are even then dramatically abolished by reticular stimulation (50, 51, 52). The suggestion is made that these responses represent further elaboration of sensory signals at a higher hierarchical level than the primary receiving areas, at a level commonly assigned to perception and gnosis.

While these researches are as yet in a preliminary stage they do point to the fact that at least some neurophysiologists, including this reviewer, look upon the cerebral cortex, and not the so-called centrencephalic system, as the site of the higher integrative functions. The cortex is a region well equipped by an avalanching phylogenetic development with an intricate structure well suited for complex functions. The overwhelming weight of behavioral and clinical observation and comparative anatomical facts support this point of view; it is the job of the physiologist to establish its truth.

BOOKS

A comprehensive and definitive monograph by Dow and Moruzzi, *The Physiology and Pathology of the Cerebellum* has appeared (95). Other books and monographs of interest are as follows: Ashby's *An Introduction to Cybernetics* (11), Hess' *Hypothalamus and Thalamus—Documentary Pictures* (in English and German) (142), Sholl's *The Organization of the Cerebral Cortex* (235), Wolstenholme and Millar's *Extrasensory Perception, A Ciba Foundation Symposium* (260), Chatfield's *Fundamentals of Clinical Neurophysiology* (65), Davson's *Physiology of the Ocular and Cerebrospinal Fluids* (82), Windle's *Regeneration in the Nervous System* (259), Smythies' *Physiological Aspects of Neurology* (237), and Field's *Brain Mechanisms and Drug Action* (105). A colloquium on the history of neurophysiology has been published in several successive issues of the *Journal of Neurophysiology* (107).

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VISCERAL FUNCTIONS OF THE NERVOUS SYSTEM^{1,2}

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RECEPTORS AND VISCERAL AFFERENTS

Taste and smell.—Chemoreceptor function in mammals and lower forms has been reviewed by Dethier (1). In frogs, Ottoson (2) recorded a slow, negative potential of 2 to 3 mv. from the pigmented portion of the nasal mucosa when a puff of odorized material (usually butanol) was blown over it, and believes that this potential represents receptor activity. The potential was not altered by cocaine to the point of blocking olfactory-bulb responses, but was promptly reduced or abolished when small quantities of ether or chloroform were blown on the mucosa, presumably because these agents injure the olfactory hairs. Walsche (3) described three types of single-unit discharge recorded with micropipettes in the rabbit olfactory bulb. Class I units discharged spontaneously and continuously, while class II units fired in rhythmic bursts synchronous with the passage of air through the nostrils; neither discharge pattern was affected by odorous substances. Class III units, although sometimes showing "spontaneous" activity, were clearly affected by odorous materials; some units showed some specificity to particular odors. In a Ferrier lecture, Le Gros Clark (4) described his anatomical investigations of the olfactory system, and speculated about the mechanism of olfactory discrimination. In pigeons, Calvin *et al.* (5) could not establish an avoidance-conditioned response to a "cheap penetrating perfume" although the birds learned rapidly when light was the conditioned stimulus. Liljestrand & Zotterman (6), using cats, recorded the responses of single chorda tympani fibers to sodium hydroxide applied to the tongue. Discharges occurred in "water fibers," "salt fibers," and to some extent, in "quinine fibers"; these workers concluded that the alkaline taste results from stimulation of several submodalities. Cohen *et al.* (7) confirmed Patton & Amassian's (8) localization of the cortical receptive zone for afferent discharges in the chorda tympani, but on the basis of latencies and thresholds, concluded that most of the primary cortical response there is mediated by tactile fibers. However, five cortical cells in that region responded only to gustatory stimulation; each of the five responded to a variety of solutions, showing none of the stimulus specificity of the receptors. Nearby units responded only to tactile stimulation of the tongue; thus, it appears that the cortical representations of taste and tactile stimulation of the tongue overlap extensively, as suggested by Patton & Amassian.

¹ The survey of literature pertaining to this review was completed in July, 1957.

² The following abbreviations are used in this chapter: CER (conditioned emotional response); DFP (diethylfluorophosphate).

Vagal and splanchnic receptors and afferents.—The carotid sinus in the hedgehog (9) and the aortic body in the cat (10) have been investigated histologically. In feline vagal fibers, Diamond & Howe (11) recorded activity originating in the aortic bodies near the root of the left subclavian artery, proving that these structures, like the bodies beneath the aortic arch, contain chemoreceptors. Bartels & Witzler (12) studied the sensitivity of the cat's carotid body to carbon dioxide. The threshold lay between 20 and 30 mm. Hg PCO_2 , and the discharge rate was linearly related to the PCO_2 , the slope being 30 impulses per sec. for each 10 mm. Hg change in PCO_2 .

A number of studies indicate that chemical agents sensitize or excite end organs innervated by vagal afferent fibers. Pulmonary and gastric stretch receptors and atrial receptors were excited by veratrum alkaloids, simultaneously becoming less sensitive to natural stimuli (13). Trichlorethylene sensitized pulmonary stretch endings to lung inflation, but did not excite them. Veratrum-induced discharges were reduced or abolished by calcium chloride, and were reinstigated by sodium citrate. Veratrum appears to act on the nerve endings, and veratrum-evoked vagal discharges apparently account for the systemic arterial hypotension following injections of these alkaloids. Rose & Lazaro (14) could produce this phenomenon by injecting veratrum into the pulmonary arteries of dogs maintained with a left ventricular pump of fixed output so that the drug did not enter the systemic circulation. The effect was abolished by cervical vagotomy.

Deflation-sensitive pulmonary receptors were not affected by veratrum, but were sensitized by phenyl diguanidine (15). They were also sensitized and excited by insufflation of ether, trichlorethylene, or chloroform. The latencies of these effects suggested equal accessibility of these receptors to the pulmonary circulation and to gases in the lungs, the most likely location being in the respiratory bronchioles and more distal parts of the respiratory tree. Paintal (15) presents evidence that these receptors mediate a reflex bradycardia.

The carotid and aortic baroreceptors are similarly susceptible to sensitization by volatile anesthetics (16, 17). Perfusion of the isolated carotid sinus with Tyrode's solution containing ether or chloroform, or sudden insufflation of these agents into the lungs, caused reflex hypotension and a bradycardia that was abolished by baroreceptor denervation.

By punctate probing of the endocardial surface while recording from isolated vagal afferent fibers, Coleridge *et al.* (18) located receptors in the junctional regions between the venae cavae and the right atrium, and between the pulmonary veins and the left atrium. Silver-stained sections from these regions showed endocardial end organs, flattened plates parallel to the endocardial surface, supplied by branched, myelinated fibers 3 to 10 μ in diameter. Henry & Pearce (19) believe the left atrial receptors regulate circulating blood volume. Their discharge varied with atrial volume, not pressure, and maneuvers causing diuresis in intact dogs caused increased firing of the atrial receptors. Also, cooling the vagus to a temperature which

blocked the afferent fibers supplying the left atrial receptors blocked the diuretic response to intra-atrial balloon distention or to negative pressure breathing. As atrial receptors are sensitive to veratrum, it would be interesting to know whether veratrum causes diuresis.

Davis *et al.* (20) found that the discharge rate of slowly adapting pulmonary stretch receptors and the total transpulmonary pressure varied with the volume, rate, and sign of lung inflation. Acceleration of volume did not affect discharge frequency. The receptors provide signals related to the work of breathing. The distention-sensitive receptors in the esophagus, the stomach, and the intestines are supplied by vagal C fibers with conduction velocities of 1.3 m. per sec. or less (21). Perhaps partially reflecting smooth muscle tone, the discharge rate was greater with rapid than with slow distention. The receptors apparently located in the muscle coats of the gut, responded to active contraction of the muscle, suggesting a "series" arrangement with contractile elements. Afferent fibers in the rat's superior laryngeal nerve supply the slowly adapting stretch receptors in the cervical esophagus. These receptors show a continuous resting discharge uninfluenced by esophageal pressure, but respond to a bolus by increasing their discharge (22).

In the cat the cervical vagus nerve contains about 30,000 fibers, 16 per cent of which are myelinated. Afferents constitute 20 per cent of the total; their cell bodies are in the vagal ganglia. The abdominal vagus is composed largely of unmyelinated afferent fibers (23). In guinea pigs, Oberholzer *et al.* (24, 25) studied the respiratory effects of vagotomy and of stimulating electrically the central end of the vagus. Three different strength-duration curves were determined for weak inspiratory, expiratory, and tonic-inspiratory effects. The last appeared to be mediated by smaller, higher-threshold fibers than the first two effects. In horses, King (26) traced the cervical course of the depressor nerve. The fibers run with the cervical vagus to the origin of the cranial laryngeal nerve, where they become diverted to pass along the plexus which forms at this site.

Downman & Evans (27) traced the spinal path of the splanchnic afferent fibers, and confirmed Amassian's (28) observation that many of the large A beta afferent fibers supply mesenteric Pacinian corpuscles, and traverse rapidly conducting (26 to 55 m. per sec.) pathways in the ipsilateral dorsal columns. The spinal path is compact, and in the cervical cord is ventral to hindlimb afferents and medial to forelimb afferents. In addition, bilateral responses (20 to 30 m. per sec.) were recorded diffusely in the anterolateral columns. The ventrolateral path is intermingled with hindlimb somatic afferents, but is distinct from the dorsal spinocerebellar path.

BULB

Conventionally, the bulbar respiratory centers of cats, determined by electrical stimulation, are pictured as occupying the caudal half of the medulla beneath the floor of the fourth ventricle, the expiratory center being dorsal and rostral to the inspiratory center. Within this region, however,

Baumgarten *et al.* (29) were unable to record unit activity synchronous with respiration. Units firing during inspiration lay lateral to the floor of the fourth ventricle, just ventral to the tractus solitarius, and units with expiratory rhythms, 2 to 3 mm. caudal to the obex, near the caudal part of the nucleus ambiguus. Rhythmic discharge patterns persisted following succinylcholine-induced respiratory paralysis which eliminated the possibilities of movement artifacts and of mistaking vagal afferent fibers for respiratory center units. Work in the author's laboratory, in collaboration with Nelson and Young, essentially agrees with these results. The respiratory patterns of cats with various brain stem transections have been studied further by Brodie & Borison (30, 31). Injection of cyanide or electrical stimulation of the floor of the fourth ventricle 3 to 6 mm. rostral to the obex and 3 mm. from the midline in vagotomized, low-decerebrate animals converted apneustic breathing to the gasping pattern typical of the medullary preparation. In medullary preparations, stimulation at the same site accelerated the rate of gasping. An apparent interaction of the respiratory and vasomotor centers was revealed by Joels & Samueloff (32), who found persisting sinus arrhythmia and arterial pressure fluctuations synchronous with respiration in dogs paralyzed with succinylcholine and maintained by diffusion respiration. Thiopentone anesthesia depressed the respiratory center, and abolished the arterial pressure fluctuations.

The role of the area postrema as a chemoreceptor trigger zone for emesis has been investigated further. In monkeys, vomiting following exposure to x-irradiation was prevented both by selective lesions of the area postrema and by subdiaphragmatic vagotomy (33). Similar lesions did not prevent the emetic action of pilocarpine in cats, but, surprisingly, destruction of the orbital cortex of the frontal lobes did (34). Clemente *et al.* (35) recorded augmented electrical activity near the area postrema after injecting hypertonic salt solutions intravenously or into the carotid or vertebral arteries, a finding suggesting an osmoreceptor function.

Anderson & Berry (36) evoked electrical activity in the bulb by stimulating the vagus or its branches. Responses to stimulation of the aortic depressor nerve were recorded in the tractus solitarius and the sensory nucleus of the vagus. Stimulation of the vagus distal to its superior laryngeal branch evoked activity in the tractus solitarius and the ipsi- and contralateral vagal commissures. Antidromic potentials were recorded in the dorsal motor nucleus of the vagus and in the nucleus ambiguus. Reflex discharges in the vagus, often associated with slowing of the heart, followed stimulation of the trigeminal nerve (37).

HYPOTHALAMUS AND BRAIN STEM

Anatomy and development.—Guillery (38) studied in detail the connections of the rat's hypothalamus by tracing degeneration (Nauta stain) following discrete brain lesions. Also in the rat, Cohn & Richter (39) determined the enzyme content of the hypothalamus during maturation. From birth, phos-

phomonoesterase I and II activity rose, to reach peak values between 10 and 15 days, and then decreased to a plateau by 40 days. Acetylcholinesterase reached maximal activity at the fifteenth day, considerably earlier than in the cerebral cortex (sixtieth day). In dog, cat, and man, Knoche (40) discerned an unmyelinated tract originating from the rostral optic chiasm and terminating in the lamina terminalis, suprachiasmatic region, paraventricular nuclei, infundibular tuberal nucleus, and the posterior lobe of the hypophysis.

Hypothalamus and adenohipophysis.—Considerable interest still centers around the effect of hypothalamic lesions on the secretion of adenohipophysial hormones. The interrelationships between the adrenal cortex and the sympathetic nervous system have been reviewed by Ramey & Goldstein (41). According to George & Way (42), lesions of the median eminence prevent ascorbic-acid depletion of the rat adrenal cortex following administration of aspirin. Greer & Erwin (43) present evidence of separate hypothalamic centers governing TSH and ACTH secretion. Amphenone caused adrenocortical and thyroid hypertrophy, presumably by blocking secretion by these glands and thus inducing increased ACTH and TSH secretion. Thyroid hypertrophy was blocked by anterior hypothalamic lesions, but amphenone still induced adrenal hypertrophy. Adrenal hypertrophy was sometimes blocked by median eminence destruction. Anterior hypothalamic lesions also prevented thyroid hypertrophy in response to propylthiouraea (44, 45) or to partial thyroidectomy (46), but the increase in iodide trapping which follows the latter operation was not prevented. Anterior hypothalamic lesions which blocked production of goiter by goiterogenic agents were compatible with normal gonadotrophin secretion as judged from testis weight and histology and from bioassays of pituitary tissue (45). Neodecortication did not alter the goitrogenic efficacy of propylthiouraea (47).

The hypothalamus may control secretion of the pituitary luteinizing factor (48). Lesions of the ventromedial nuclei led to vaginal cornification in female rats, and the ovaries contained follicles of all sizes but no corpora lutea. Luteinization occurred readily when human chorionic gonadotrophin and pituitary interstitial-cell-stimulating hormone were administered. The electrical pattern recorded through electrodes implanted in the anterior and lateral hypothalamus around the medial forebrain bundle was altered by vaginal stimulation in estrous cats, but not in anestrous animals (49). In male rats, injection of testosterone through implanted pipettes in the medial preoptic area elicited maternal behavior; similar injections into the lateral preoptic region resulted in male sexual behavior (50).

The role of the portal vessels in hypothalamohypophysial relationships has been the subject of several investigations. Engelhardt (51) described in detail the hypothalamohypophysial angioarchitecture in the cat, dog, and monkey, and Glydon (52), the developmental anatomy of the rat's portal vessels. After stalk section, the volume of the pars distalis in the rabbit diminished by 68 to 83 per cent, while the neurohypophysis shrank to 26 to

27 per cent of its normal volume. Prevention of portal regeneration did not increase the atrophy, so it seems unlikely that ischemia underlies the adeno-hypophyseal atrophy (53). When portal regeneration was prevented, stalk section in rabbits blocked lymphopenic responses to restraint and cold exposure, but not those to laparotomy or injected epinephrine; adrenal ascorbic-acid depletion still followed unilateral adrenalectomy (54). Similarly, stalk and portal vessel interruption reduced thyroid responsiveness (inhibition of I^{131} release) to such stimuli as restraint and stilbestrol, but not that to laparotomy and thyroxine (55).

McCann (56) presents the thesis that the antidiuretic hormone is the chemical mediator between the hypothalamus and the ACTH-secreting pituitary cells. Rats under pentobarbital anesthesia, or with hypothalamic lesions preventing adrenal ascorbic-acid depletion in response to stress, responded to injections of pitressin. The degree of ascorbic-acid depletion was proportionate to the pressor activity of the extracts. However, Greer & Erwin (43) observed adrenal hypertrophy following amphenone administration in rats with lesions of the supraopticohypophyseal pathway sufficient to cause marked diabetes insipidus. According to De Groot (57), the Gomori-positive neurosecretory material in the supraopticohypophyseal system is unrelated to gonadotrophin release since normal secretion persisted after the neurosecretory material was depleted by dehydration. Sutin & Clemente (58) observed increased electrical activity in the region rostral and lateral to the mammillary bodies when pitressin was injected intravenously.

Transplantation of the adrenal to the thigh did not reduce aldosterone secretion (59). Since aldosterone secretion diminished in decapitate and decerebrate preparations, but not in decorticate animals or in those surviving combined transections of the spinal cord, the sympathetic trunks, and the vagi, Rauschkolb & Farrell (60) conclude that a humoral substance generated in the diencephalon regulates aldosterone secretion.

Neurohypophysis and water regulation.—The histology of the neurohypophysis has been studied with both light (61) and electron microscopes (62). Duncan (62) observed neurosecretory droplets, 0.1 to 0.2 μ in diameter, in myelinated and unmyelinated fibers, but not in the pituicytes, perivascular spaces, or vessels in the chicken's posterior lobe. A limiting membrane separates both the nerve fibers and the pituicytes from the perivascular spaces, posing a problem about the mechanism for transferring neurohumors from nerve fiber to blood vessel.

German workers have studied exhaustively the Gomori-positive diencephalic-hypophyseal system in lungfish (63), frogs (64), a plant bug (65), Rhode Island red hens (66), rats, guinea pigs, dogs, and humans (67). The amount of Gomori-positive neurosecretory material was reduced or depleted by dehydration, cortisone, pituitrin (57), tourniquet shock (68), injected diencephalic lipoid extracts (69), ether anesthesia, and "stress situations involving pain, fear, or rage" (70). Providing rats with 2.5 per cent sodium chloride as drinking water for 8 to 10 days increased the acetylcholinesterase

content of the supraoptic and paraventricular nuclei (71). Secretion of oxytocic hormone is thought by Abrahams & Pickford (72) to depend on a cholinergic mechanism because anticholinesterases injected into the supraoptic nuclei of dogs pretreated with stilbestrol caused increased uterine contractions. In dogs, Zuidema & Clarke (73) have confirmed Verney's well-known observation that intracarotid injection of hypertonic saline causes antidiuresis, and Friedman *et al.* (74) found this response to be greater than normal in rats with senile hypertension or made hypertensive by desoxycorticosterone. Dicker & Nunn (75) question whether the antidiuretic hormone causes the oliguria of thirsting rats; vasopressin did not affect urinary excretion by either satiated or thirsting rats.

A hypothalamic mechanism for water seeking and ingestion, as opposed to water conservation, has been described by Andersson & McCann (76). In goats, electrical stimulation of the region between the columns of the fornix and the tract of Vicq d'Azyr caused polydipsia leading to overhydration up to 40 per cent of the body weight, followed by hemal dilution and polyuria. Microinjections of hypertonic saline into the same region also caused polydipsia. Attempts to condition drinking to light or sound signs when hypothalamic stimulation was the unconditioned stimulus failed (77). The experiments of Miller *et al.* (78) indicate peripheral mechanisms for thirst satiation in rats. Water introduced into the stomach via a fistula reduced both (a) bar pressing for a small aperiodic water reward, and (b) the amount of water consumed to satiation. When the same amount of water was given by mouth, the test scores were even lower. There thus appear to be satiation receptors in both the mouth-throat and the stomach-intestine regions. Williams & Teitelbaum (79) describe a method of controlling rats' drinking behavior by an operant-conditioning technique—the rats delay a painful shock by licking the drinking tube. Rats run for 20 days with a nutrient fluid became obese.

Hunger.—Mayer (80) has reviewed the relation of the hypothalamus to hunger and food drive, and presented evidence for his "glucoreceptor" theory of hypothalamic feeding and satiety centers. In subjects who ingested amino-acid mixtures or glucose solutions, appetite was correlated with both low venous blood sugar and low arteriovenous glucose differences (81); these results are consistent with Mayer's theory. Following on a previous demonstration that gold thioglucose produces specific lesions in the ventromedial nucleus of the mouse (82), Mayer & Marshall (83) have now found that, of a large number of gold-thio compounds tested, only thioglucose produces hypothalamic lesions and obesity. Since the general toxicity of many of the other compounds was about the same as that of the effective dose of gold thioglucose, Mayer & Marshall suggested that the affinity of "glucoreceptors" in the ventral medial nuclei for the glucose moiety of the compound causes them to accumulate damagingly high quantities of gold. An interesting consequence is that, if the argument proves correct, regulation would appear to depend primarily on the "satiety center" in the ventromedial nuclei rather

than on the more laterally situated "feeding center," for the latter is not damaged by gold thioglucose. Gold thioglucose-obese mice do not increase their food intake when the diet is diluted nor in accordance with exercise (84) or cold exposure (85); they run less than normal mice do (85), and eat larger quantities of food but less frequently than normal mice (84). Unlike yellow mice, gold-thioglucose-obese mice exhibit abnormal activity patterns in response to fasting and refeeding, a difference suggesting to Hollifield & Parson (86) that yellow mice have intact hypothalamic feeding centers. In three types of obesity (hypothalamic, hereditary-hyperglycemic, gold-thioglucose) the liver, heart, and kidneys are enlarged (87), and intestinal absorption of glucose is above normal (88). These appear to be nonspecific consequences of obesity. Hereditary-hyperglycemic-obese animals have enlarged pancreases and thymuses and subnormal brain weights, changes which may be specific parts of the syndrome of which obesity is also a component (87). In hypothalamic-obese rats, Montemurro & Stevenson (89) examined body composition and extracellular fluid space. The only major change in gross body composition was an excess of fat (50.8 per cent versus 14.4 per cent for normals); extracellular fluid volume (inulin space) was small relative to body size.

The relationship of eating to water intake was reinvestigated by Lepkovsky *et al.* (90); thirsting rats reduced their food intake. The stomach contents of thirsting and watered rats contained the same proportion of water, the extra water in thirsty animals probably being drawn from the skin and, possibly, adipose tissues. These authors believe that rats eat in accordance with available water in the body.

Amphetamine given to cats caused increased electrical activity of the ventromedial hypothalamic nucleus (91), but, according to Andersson & Larsson (92), this appetite-reducing drug does not directly affect the hypothalamus. In dogs, amphetamine-induced inhibition of eating and of drinking following hypertonic saline solutions was abolished by section of the fore-brain anterior to the orbital sulcus. These workers believe that amphetamine acts through the frontal lobes to affect the hypothalamic thirst and hunger centers.

Cardiovascular and temperature regulation.—Anterior hypothalamic stimulation in cats caused hypotension and bradycardia which were abolished by vagotomy; posterior hypothalamic stimulation caused ventricular extrasystoles, shifting of pacemaker, changes in the T wave, and elevation of arterial pressure (93). Kundt *et al.* (94) measured simultaneously the hypothalamic temperature (by means of an implanted thermocouple) and the ear blood flow in cats. If the feet were immersed in cold water, skin blood flow decreased, and hypothalamic temperature increased; opposite changes were induced by warming the feet. The changes in blood flow preceded those in hypothalamic temperature, suggesting that peripheral rather than central thermostatic mechanisms alter skin blood flow.

In decorticate cats, panting followed transections 1 mm. rostral to the

chiasm; additional sections at the level of the tuber cinereum abolished panting, a sequence indicating a panting center between these two levels (95). Electrically stimulating the dorsal half of the area between the anterior commissure and the optic chiasm in unanesthetized goats resulted in panting and vasodilation in the ears; lowering the body temperature raised the threshold (96).

In cats, lesions in a pathway extending from the lateral hypothalamus through the portion of the brain stem ventrolateral to the red nucleus and through the lateral spinal columns abolished shivering; the pathway is neither pyramidal nor reticulospinal (97). Within this pathway, stimulation elicited a tremor resembling shivering (98), and recorded unit activity was accelerated by cooling and abolished by heating the animal (99). Isolating the units from the ascending neural inputs did not affect the discharge. Boyarsky & Stewart (100) inhibited shivering in cats by electrically stimulating the skin or the peripheral nerves; the inhibitory pathway ascends in the dorsal half of the cord.

Bladder and gut.—Two investigations combining cystometric studies with brain stem transection and discrete brain lesions have revealed a hitherto unsuspected neural hierarchy of bladder control (101, 102). There is a facilitatory region in the mammillary region, and an inhibitory center has been found in the tegmentum lateral to the central gray at the superior collicular level; Barrington's facilitatory center has been localized at the isthmus level, ventral to the lateral angles of the periventricular gray.

Chronic hypothalamic stimulation through implanted electrodes produced ulcers or other lesions of the gut in 8 of 19 monkeys (103). Effective stimulus sites were near the midline at the preoptic, suprachiasmatic, and mammillary levels.

CEREBRAL CORTEX AND VISCERAL REGULATION

Most contemporary interest in this field centers on the rhinencephalon and the temporal cortex. Whitlock & Nauta (104) have published a careful study of the subcortical projections from the monkey temporal lobe that will be useful to those who need descending pathways to explain physiological findings. Cragg & Hamlyn (105) examined electrophysiologically and histologically the commissural and septal connections of the rabbit hippocampus, and Adey *et al.* (106, 107) have made similar studies on the entorhinal area of the phalanger.

Seizure discharges in the amygdala were studied by Gloor (108) and by Tokizane & Sawyer (109). The latter authors found that insulin-induced hypoglycemia in rabbits caused highly localized seizure discharges in the amygdala, the hippocampus or both. These discharges were not accompanied by somatic motor activity. More severe seizures sometimes projected to the preoptic, the hypothalamic, and other brain stem regions without reaching the frontal limbic cortex. It is suggested that the beneficial effects of insulin therapy are related to undetected subcortical seizures. Stimulating the in-

sula caused shivering, piloerection, and, sometimes, salivation and inspiratory apnea in monkeys (110). Anand & Dua (111) describe the respiratory and cardiovascular effects of stimulating limbic structures in cats and monkeys. MacLean *et al.* (112) recorded responses in the pyriform lobe after electrically stimulating the olfactory fila, bulb, and tract. Conduction velocity in the fila was estimated at 1.4 m. per sec., and responses to fila stimulation were potentiated following repetitive stimulation of the olfactory bulb or tract.

A vasodilator pathway originating in the motor cortex of the dog and cat was described by Lindgren *et al.* (113), and Langford *et al.* (114) described vasoconstriction resulting from motor cortex stimulation in cats. Generalized seizures elicited in curarized unanesthetized dogs were associated with vasoconstriction and arterial pressure elevations. Apparently, involvement of the frontal poles is a necessary condition for the development of hypertension (115). Ferguson *et al.* (116) found that stimulation of the anterior sigmoid gyrus induced contraction of the denervated nictitating membrane and increased adrenomedullary secretion. Stimulating vasopressor cortical foci caused relaxation of the contracted nictitating membrane, and this effect was not altered by vagotomy or atropinization. The authors conclude that stimulation of cortical depressor foci inhibits epinephrine secretion.

THE BRAIN AND BEHAVIOR

With knife and with stimulating electrode that behavioral bastion, the amygdala and the pyriform lobe, is repeatedly attacked (106, 107, 117 to 120). The invaders can scarcely claim a decisive victory, but a Trojan horse may yet be devised. Generally, lesions of the amygdala, of the pyriform cortex or both cause a greater tameness and a loss of aggressive reactions [see ref. (106), (118), (119), (120)]. In monkeys, amygdectomy slowed acquisition of a conditioned avoidance response, but did not affect retention of a previously acquired response although extinction was more rapid than in intact animals (118). Such an operation did not alter sexual behavior in monkeys (118), dogs (119), or cats (120); the dogs' food intake was temporarily (two weeks) elevated (119). Stimulation of the amygdala in waking rabbits caused sniffing and licking of the perineum; injected gonadotrophins intensified these effects (117). Similar stimulation in the cat (120) caused cowering or attentive states when the stimulus sites were basal and lateral, and rage reactions when the sites were central and medial. Gastric secretion and motility were increased, and, in hormonally primed animals, ovulation and uterine contractions were induced. Brady has reviewed the literature on the role of the paleocortex in behavioral motivation (121) and the effect of drugs on behavior and emotional activity (122).

Beach *et al.* (123) found that unilateral neodecortication caused defects in the mating behavior of cats, and that bilateral decortication completely abolished all mating behavior except its most undesirable component, vocalization. Studies of partial decortication indicate that frontal lesions impair

matings by interfering with motor performance (124), while parietal and temporal lesions have slight effects referable to the accompanying sensory deficits (125).

Hunsper (126) has mapped the subcortical centers which, when stimulated, produce emotional behavior in unanesthetized cats. Affective defense reactions were elicited by stimulating the perifornical region in the rostral hypothalamus or the middle portion of the central gray in the midbrain; strong stimulation resulted in directed attacks. Stimulating nearby regions elicited hissing with flight. Using hypothalamic stimulation as the unconditioned stimulus, Ban & Shinoda (127) established conditioned respiratory, gastrointestinal, and pupillary responses in rabbits. In monkeys, Delgado *et al.* (128) studied centrally evoked fear responses. Simulation of some regions elicited responses by which the animal had previously learned, to avoid painful shock. Positive sites were the medial amygdala, the rhinal fissure, the Gasserian ganglion, the rostral pons, the central mesencephalic gray, the posteroventral thalamic nucleus, the external medulla, and the lamina of the pallidum. Negative sites were the sensorimotor cortex, the ventrolateral thalamic nucleus, the pulvinar, the putamen, the substantia nigra, the tegmentum, the anterior hippocampus, and the posterior amygdala. In rabbits, Visser *et al.* (129, 130) established conditioned bradycardia, a whiff of ammonia being the unconditioned stimulus. When unreinforced, the conditioned bradycardia occurred mysteriously at the exact time the reinforcing signal would have occurred.

Rats placed in a conflict situation (approach-avoidance) for thirty days have a high incidence of gastric ulcers; fear situations (aperiodically paired light and shock) also increased gastric lesions (131). The psychological variables involved have been analyzed by Sawrey *et al.* (132). Cats exposed to barking dogs showed relative anemia of the renal cortex (133). Eosinopenic reactions in humans exposed to stressful situations were studied by Wagner (134) and by Markkanen *et al.* (135). Evaluation of the vegetative structure of individuals has been attempted (136, 137, 138).

Brady has published a series of reports (139 to 143) summarizing the Walter Reed group's work on emotional behavior. Most of the experiments are based on his ingenious conditioned emotional response (CER) technique. In this method the rate of bar pressing for a reward is measured. While the animal is working, a buzzer is sounded some minutes before a brief painful shock is administered. After repeated presentations of the buzzer and the shock, the sound of the buzzer acquires significance as a signal of impending shock, and this significance is reflected by a decrease in the rate of bar pressing while the buzzer is on; the animal crouches in the corner and appears apprehensive. After the shock is delivered the animal resumes bar pressing at the former rate. The change in slope of the integrated bar-pressing recording when the buzzer sounds is a measure of the intensity of the CER. In cats, rats, and monkeys, CER was readily established when the animals were bar-pressing for food or water, but not when intracranial

self-stimulation was the reward (143). Repeated (14 to 21) electroshock convulsions abolished previously established CER's; fewer (9, 10, 11) treatments attenuated but did not abolish the responses (142). Reserpine abolished CER in rat and monkey, and blocked the increased plasma 17-hydroxycorticosteroid levels which occur in untreated animals (139, 140).

GANGLIA AND AUTONOMIC EFFERENTS

Ganglia and nerve components.—The metabolism of the sympathetic ganglion cells has been reviewed by Larrabee & Horowicz (144). The ultrastructure of the synapses in the rabbit's superior cervical ganglion was explored with an electron microscope; presynaptic and postsynaptic membranes were separated by about 200 Å (145). In the same species, an accessory ganglion half-way between the ansa subclavia and the superior ganglion is innervated by preganglionic B fibers ascending in the cervical trunk; some continue up to innervate cells in the superior cervical ganglion (146). In man, a ganglion at C₇ was found more commonly than the classically described middle cervical ganglion at C₆ (147). Appleton & Waites (148) have described an approach to the superior cervical ganglion in sheep. In the cat, partial preganglionic denervation of the superior cervical ganglion was followed by sprouting of new terminals from the remaining fibers (149). After the rami T₁ to T₃ were severed, 90 per cent of the preganglionic input to the ganglion degenerated, but stimulation of the remaining trunk four to eight weeks later caused maximal contraction of the nictitating membrane. New connections in the ganglion were demonstrated histologically.

The components of the vagus nerve of man (150) and of the cat (23) have been investigated. In the cat (see also the discussion of afferents above), the efferent fibers in the cervical vagus derive almost entirely from centrally situated cell bodies and include all fibers greater than 12 μ and about half of those which are less than 6 μ in diameter. The recurrent laryngeal nerve has a unimodal fiber distribution with a peak at 10 to 12 μ , the absence of proprioceptors in the intrinsic laryngeal muscles being associated with absence of a gamma heap (151). In both the cat and man there are myelinated postganglionic fibers in the gray rami communicantis (152, 153) and unmyelinated postganglionic fibers in the thoracic and lumbar splanchnic nerves (154). The unmyelinated fibers derive from numerous small ganglia situated along the course of the nerve.

Head and eye.—Functional reinnervation of the nictitating membrane by cholinergic fibers from the hypoglossal nerve was described by Vera *et al.* (155). The new endings were blocked by atropine, but were not affected by *d*-tubocurarine or dibenamine. Neuromuscular endings in the form of delicate terminal rings on the ciliary muscle of man, cat, bull, dog, and goat were described by Génis-Gálvez (156). Pupillary responses to iterative stimulation of the cervical sympathetic chain was studied in patients undergoing surgery (157). Frequency-response curves were hyperbolic, with near maximal response to 10 per sec. Stark & Sherman (158) analyzed the consensual pupil-

lary reflex to light as a servomechanism; the system has a low gain and is extremely stable.

Heart and lungs.—The courses of the dog's cardioinhibitor fibers (159) and the sheep's cardioaccelerator fibers (160) have been described. Stimulation of the peripheral end of the divided canine vagus increased coronary flow, the increase being blocked by atropine (161). Stimulating the central vagal stump or clamping the carotid also caused coronary dilation. Using refined methods to evaluate cardiac function in intact dogs, Anzola & Rushmer (162) found that stimulating the cardiac sympathetic nerves caused tachycardia, systemic hypertension, and increased systolic ejection [see also (163)]. Cardiac changes during exercise resembled more closely those produced by sympathetic nerve stimulation than those resulting from injection of catechol amines (164). This finding suggests that direct neural influences are more important than humoral in the determination of cardiac responses to exercise. Similar conclusions were reached by Folkow *et al.* (165).

The vagal innervation of the mouse's lung was described by Honjin (166, 167). In dogs, the lung edema produced by intracisternal injection of veratrine was accompanied by a shift of blood from the systemic to the pulmonary circuit; when the systemic circulation was mechanically excluded, no lung edema occurred (168). Pulmonary arterial pressure still rose, and changes in lung opacity indicating vasoconstriction were noted, suggesting that pulmonary vasomotor reflexes contribute to the production of edema. Ferguson & Berkas (169) believe that a vasoconstrictor reflex protects the pulmonary capillaries from excessive arterial pressures; lung denervation increased the susceptibility to lung edema in dogs subjected to high pulmonary pressure and flows through a controlled systemic arterial shunt. Spinal transection protected rats from the lung edema induced by epinephrine injection (170) or by preoptic lesions (171). The latter type of lung edema is prevented by splanchnic nerve section, but not by adrenal demedullation (172).

Skin and blood vessels.—Niebauer (173) described the histology of the sympathetic endings in cutaneous structures. Bárány & Cooper (174) noted patchy distribution of sweating in diabetic patients. In nonsweating areas, neither electrical stimulation of the skin nor local, intradermal, acetylcholine injections caused sweating or piloerection as they do in normal or in preganglionically sympathectomized individuals, a fact suggesting that diabetics have some defect in postganglionic pilomotor and sudomotor innervation. In horses, section of the cervical sympathetic trunk is followed by spontaneous sweating about the face; stimulation caused inhibition of epinephrine-elicited sweating (175).

The blood vessels of the joints receive vasoconstrictor innervation through the articular nerves; constrictor tone is diminished by carotid occlusion (176). In the dog's footpad, which contains numerous arteriovenous anastomoses, Green *et al.* (177) found no evidence of vasodilator innervation; stimulation of the lumbar sympathetic chain caused constriction of the small

arteries but also of the small veins (178). In man, Gaskell (179) could not demonstrate vasodilator innervation of the hand; neither anesthetizing the nerves in heated subjects nor injecting atropine intra-arterially altered blood flow. Roddie *et al.* (180, 181) also failed to find vasodilator innervation of the hand, but present evidence (based on plethysmographic and venous oxygen saturation determinations) for a cholinergic vasodilator system supplying the forearm. In intact dogs, but not in those after lumbar sympathectomy, rewarming a leg exposed for 3.5 hr. to -4°C . caused the blood flow to rise above that in the contralateral, unexposed leg, indicating a paralysis of vasomotor nerves after cold exposure (182). Ahmad (183) described a "die-away" response in humans, a decrease in forearm blood flow during the second hour of immersion in water at 41°C .; this response is not altered by sympathectomy, and is thus independent of the vasomotor centers and the sympathetic nervous system. Löfving & Mellander (184) found considerable residual tone in skeletal muscle vessels following sympathectomy even though the skin vasculature was maximally dilated; these authors believe the residual tone to be a property of the smooth muscle rather than a persisting sensitivity to circulating vasoconstrictor substances.

Abdominal and pelvic structures.—Nerve endings in the intestine (185, 186), testis (187), and the erectile cavernous tissue (188) have been studied histologically. Retrograde degeneration of the ganglion cells in the cat's inferior mesenteric ganglion was investigated by Acheson & Schwarzsacher (189); electrical studies indicated persisting synaptic function in rather severely chromatolyzed cells. Stimulating the canine pelvic nerve caused detrusor contraction in the contralateral as well as the ipsilateral half of the bladder; contralateral contraction patterns varied in different parts of the bladder (190). Bishop *et al.* (191) found the tone of the external anal sphincter in cats unaltered after spinal transection but abolished after pudendal nerve section. Distending the colon inhibited sphincter tone via afferent fibers in the pelvic nerves.

VISCERAL REFLEXES

Cardiovascular system.—Sharpey-Shafer (192) found that tabetic patients often had defective baroreceptor reflexes, the afferent pathways appearing to be at fault. The dog's baroreceptor reflexes were severely depressed by barbital and exaggerated by chloralose anesthesia (193), the effects of pentobarbital and ether anesthesia were intermediate between these two extremes. In dogs, carotid occlusion caused venoconstriction as well as arteriolar constriction (194), the veins also participated in the vasoconstrictor response to elevated intracranial pressure (195). In rabbits, stimulating the afferent C fibers caused hypotension with slight bradycardia, but did not affect respiration (196). Rosenberg (197) concluded that the venovasomotor reflex (increased vascular tone resulting from increased venous pressure) in rabbits is mediated by local mechanisms independent of the central nervous system. Thermally induced vasomotor responses in pa-

tients with spinal cord lesions were investigated by Cooper *et al.* (198) and by Redisch *et al.* (199). When warmed, hemiplegics showed normal vasodilation in the leg and the foot, but sympathectomized patients and paraplegics with lesions from C₅ to T₁₀ did not. Roddie & Shepherd (200) described increased forearm blood in recumbent subjects when their legs were elevated; the dilation, which appeared to be largely confined to muscle vasculature, was blocked by nerve block or sympathectomy. Hypoxia caused increased coronary flow in both intact and adrenosympathectomized dogs (201); a local metabolic rather than a neural mechanism seems to be responsible. Partial or complete occlusion of one coronary vessel caused increased flow in other coronary branches (202), no evidence of the previously postulated intercoronary vasoconstrictor reflexes was found.

Sweat glands.—Sweating patterns appeared to be independent of age, but older men (44 to 57 yr.) had slower sweating responses to thermal stimulation than did younger men (18 to 23 yr.) (203). Watkins (204) was unable to confirm the existence of a hemihydrotic reflex (decreased sweating on the side to which pressure is applied and an increase on the opposite side) described by Japanese workers. Central regulation of the galvanic skin reflex in cats was studied extensively by Wang and his coworkers (205 to 209). Decerebration abolished the reflex, apparently by leaving unopposed an inhibitory component in the bulbar reticular formation; the reflex was re-established by cooling or locally anesthetizing the bulbar ventromedial reticular system. Bulbar reticular stimulation in the intact animal inhibited the galvanic skin reflex.

NEUROHUMORS, TRANSMITTERS, AND DRUGS

A monograph by Burn (210) gives a broad summary of the pharmacology and physiology of autonomic transmitters. Pitkänen (211) described a method for determining the amount of epinephrine and norepinephrine in urine; insulin-induced hypoglycemia increased epinephrine secretion 10 to 20 times without changing norepinephrine excretion. In adrenalectomized patients, norepinephrine excretion increased when they were shifted from a reclining position to a head-up tilt of 75°; the increase was presumed to reflect increased secretion of the neurotransmitter during reflex activation of the vasomotor system (212). In man ether anesthesia increased plasma norepinephrine levels, with little increase in epinephrine (213); in dogs, plasma catechol amine concentration was increased by plane III anesthesia with ether, chloroform and divinyl ether, but not that with pentothal (214). In cats, hypercapnia induced sympathoadrenal discharge, indicated by contraction of the denervated nictitating membrane; adrenalectomy reduced this response by 60 per cent (215). Intravenously administered histamine caused contraction of the innervated nictitating membrane, apparently by a central stimulating action (216). Discharge of catechol amines from the adrenal medulla in cats and rats was associated with a proportionate decrease of its ATP content (217). Sympathetic amine content of the cat's right and left

adrenal glands did not differ significantly (218). In cats, von Euler (219) found that infusions of epinephrine or norepinephrine did not increase the catechol amine content of the heart, the liver, the spleen, the kidney, or the skeletal muscle; there is thus no evidence that these organs accumulate circulating amines administered in physiological doses. Reserpine treatment diminished the norepinephrine content of the cat's hypothalamus; there was also depletion of the amines in the innervated but not in the denervated adrenal (220).

Infusion of epinephrine and norepinephrine in dogs caused bradycardia, whereas exercise caused pronounced tachycardia (164). Cardiovascular regulation appears to depend more on direct neural mechanism than on circulating hormones (164, 165). Epinephrine, norepinephrine and isopropylartanol injected into the coronary artery caused dilation (221). Both epinephrine and norepinephrine increased the total vascular resistance in the dog's forelimb vasculature (222), and decreased the blood flow through the joints (223). Elimination of neural compensatory mechanisms conditioned arterial pressure responses to epinephrine; spinal (C_8 to T_1) vagotomized dogs showed hypertensive response to injected epinephrine three times greater than that of intact dogs (224). Cessation of norepinephrine infusion in cats caused a marked fall in arterial pressure believed to result from a partial blocking action of the drug on vasomotor synapses, reducing vasoconstrictor tone (225). In barbiturate-anesthetized dogs, ganglionic blocking agents caused decreased aortic and mesenteric arterial pressure and flow without affecting (or slightly decreasing) the calculated resistance in the mesenteric circuit (226). Epinephrine and, to a lesser extent, norepinephrine caused splenic contraction and vasoconstriction (227). Hypertension induced in rabbits by bilateral carotid ligature was reduced by hexamethonium, dibenamine, and benzodioxane; renal hypertension was reduced by veriloid and dihydroergocornine (228). The author concludes that cerebral hypertension results from an overactive sympathetic nervous system and that renal hypertension results from increased vascular reactivity to normal sympathetic tone.

Contradictory to previous reports, neither adrenomedullary secretions nor the sympathetic nervous system condition the calorogenic response to thyroxine; Surtshin *et al.* (229) found that a thyroxine-induced elevation of oxygen consumption in rats was not altered by dibenzylamine or adrenalectomy. A role of the sympathetic amines in temperature regulation is indicated by Cottle & Carlson's work (230). Cold-adapted rats, curarized to prevent shivering, maintained their body temperatures when exposed to 5°C., but warm-adapted animals did not. Adrenomedullation markedly reduced the superiority of cold-adapted animals. Hexamethonium or piperoxane completely abolished the capacity of curarized cold-adapted animals to regulate against cold; in the case of hexamethonium blockage, the response was restored by injecting norepinephrine (Hsieh & Carlson, in a personal communication).

Epinephrine and norepinephrine inhibited gastric motility in unanesthetized dogs, but augmented it in anesthetized animals; vagotomy or atropine also reversed epinephrine's effects (231, 232). Sensitization to epinephrine and other excitatory drugs followed supradiaphragmatic vagotomy or a three-day course of treatment with a parasympatholytic drug, Hö 9980 (piperidino-ethyl-diphenyl-azetamid) (232, 233). The submaxillary gland was similarly sensitized to adrenaline by Hö 9980 or by atropine, and this sensitization was more extreme than that following denervation (234).

The acetylcholinesterase activity of individual sympathetic ganglion cells of the frog and the rat was determined by Giacobini (235); values in the rat varied widely between 2×10^{-4} and 30×10^{-4} μ l CO_2 per hr. Histochemical staining revealed specific cholinesterase in endings around arteriovenous anastomoses of human digital skin and about the tubules of the eccrine sweat glands (236). Cholinesterase staining of elements in the cat stellate and ciliary ganglion was abolished by administration of diethyl-fluorophosphate (DFP); when animals were given pyridine-2-aldoxime methiodide prior to DFP, staining was comparable to that in controls (237). Intra-arterially injected histamine, pilocarpine, or 5-hydroxytryptamine augmented nictitating membrane contraction to stimulation of preganglionic fibers in the cervical sympathetic chain, but not to stimulation of postganglionic branches from the superior cervical ganglion (238).

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HIGHER CEREBRAL FUNCTIONS¹

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The past year has seen the exploitation of two important techniques which are contributing significantly to our further understanding of the higher functions of the central nervous system. The first of these involves the use of implanted electrodes in the unanesthetized animal. Experiments of this sort have involved not only the lower animals and primates, but their use in humans has also been continued, and with the development of techniques for both stimulating and recording are contributing significantly to our knowledge of the nature and location of function in the nervous system (1). These techniques have also been expanded recently by the development of microelectrodes capable of recording from single units in the unanesthetized animal.

The use of implanted electrodes has also permitted the development of chronic experiments and has been associated with the other important development, namely, the marriage of the techniques of classical psychology with those of basic physiology. Many of the classical experiments on learning, conditioning, and motivation are now being re-examined, and are being carried out under conditions in which the electrical activity of the nervous system is recorded during the process.

The third dimension is now being added to experiments in neurophysiology—that third dimension being time. The stage has been set for the assessment of slow and persistent changes in the electrical responses of the central nervous system, these changes being co-ordinated with the alterations in behavior with which they are associated.

THE RETICULAR SYSTEM AND THE MAINTENANCE OF CONSCIOUSNESS

Data regarding the anatomy of this important system have been summarized by Papez (2). This paper is in effect a summary of previous work regarding the anatomy and connections of these structures. The original data on which the conclusions are based are not presented in this summary.

According to the outline presented, afferent impulses arriving over collaterals from each of the major sensory pathways end in the brain stem reticular formation. From this formation, fibers travelling through the reticulothalamic, tegmentothalamic, and tectothalamic tracts end in the intralaminar nuclei of the thalamus. These nuclei in turn send fibers to the reticular nucleus of the thalamus. This nucleus forms a thin mantle over the other thalamic intralaminar nuclei. From the reticular nucleus of the thalamus, fibers radiate to various areas of the cerebral cortex. The reticular

¹ The survey of literature pertaining to this review was completed in August 1957.

nucleus, which lies on the outer surface of the thalamus, forms the last link in the nonspecific path to the cortex for electroencephalographic (EEG) rhythms related to general consciousness. The nonspecific neural system is distinct from specific sensory and other neural systems that activate the cortex.

Included in this paper is a more detailed analysis of the distribution of afferent and efferent connections. The author indicates that there is a specific distribution of radiations from certain of the intralaminar nuclei, passing to specific areas of the reticular nucleus, and in turn radiating to limited areas of the cerebral cortex.

In regard to the more detailed anatomy of the system, it is pointed out that, within the cortex, the nonspecific fibers from the reticular nucleus of the thalamus connect with the pyramidal and granule cells the actions of which produce the nonspecific EEG rhythms. The pyramidal cells thus activated fire back down along their axones into the thalamus. Apical dendrites, however, backfire into layer I of the cortex where they spread through many contacts with dendrites coming from granule cells of layer II. These granule cells discharge their axones into nets, which they form around pyramidal cells in layer III. The author concludes,

The facts suggest that the arrangement for backfire of apical dendrites into dendrites of granule cells in the outer layer of cortex may be the actual substance that produces consciousness.

Physiological and clinical experiments continue to indicate the significance of this entire system in relation to consciousness (3). Interesting data relative to the functional characteristics of this system are presented by Sharpes & Jasper (4). The authors recorded the EEG of sleeping cats, and observed variations in the electrographic responses of these animals to an auditory stimulus consisting of a standard tone. The stimulus was presented at irregular intervals over a period of several hours in each experiment, and variations in the character and duration of the arousal reaction of EEG pattern were noted. Repetition of a specific tone which initially produces long lasting arousal of a sleeping cat fails to do so after 20 or 30 trials. This habituation is specific in respect to the quality, modality or pattern of a given stimulus—variations in pitch for example would produce a complete arousal reaction following habituation. There were observed to be two types of arousal reaction, one of short latency and brief duration, and another of longer latency and greater persistence. The long lasting reaction was the most susceptible to habituation. Complete section of the brachium of the inferior colliculus bilaterally raised the arousal threshold and abolished the rapid phasic response. This procedure also increased the speed of habituation and impaired the tonal specificity of it. The specificity of habituation to the pitch of the repeated sound is unaffected by complete ablation of all cortical auditory receiving areas and adjacent cortical structures. Discrimination between tonal patterns, however, may be impaired by such cortical destruction.

The authors conclude that the tonic slow and persistent arousal response is probably mediated by the lower portions of the ascending reticular system which is capable of habituation selective for a given modality of sensory stimulation, but not for more highly differentiated qualities of the stimulus. The more rapid and more highly differentiated response is probably mediated by the upper portions of the activating system, particularly the unspecific thalamic projection system and adjacent sensory structures.

The exact mechanism of habituation continues to remain a mystery. The fact that cortical auditory potentials with repeated stimuli are recorded unchanged indicates that habituation of the arousal reaction does not depend upon changes occurring in the specific auditory system. It seems far more likely that the habituation takes place within the ascending reticular system, or the unspecific thalamic projection system.

Especially difficult to explain are the remarkably long latent periods occasionally observed in these experiments. It might be assumed that some activation reaction could be initiated in the mesencephalic reticular system and spread slowly—over many seconds—to the cortical mantle, but electrodes placed in the reticular formation in these experiments failed to reveal any characteristic sign of activation during the latent period preceding the onset of the cortical response. The possibility of some hormonal transmitter mechanism is discussed, but must be considered purely hypothetical in this situation.

Clinical experiences continue to demonstrate the importance of continued afferent stimulation to the maintenance of consciousness, and even to normal mental activity. Miles (5) reports observations on 42 patients undergoing spinal anesthesia. Of these 42 patients, 38 experienced the phenomenon of phantom limb during the period of time that their lower extremities were anesthetized. The author concludes that the phantom limb is a reaction of the mind to the complete sensory and motor deprivation of the lower extremities under these circumstances. A somewhat comparable experiment is that involving the deprivation of normal individuals of all sensory stimulation for protracted periods of time. A popular review of such experiments has been prepared by Heron (6). The author reports on a series of experiments designed not so much to cut off all sensory stimulation as to remove all patterned or perceptual stimulation as far as possible. College students were employed to lie on a comfortable bed in a lighted cubicle 24 hr. a day. They wore translucent plastic visors which transmitted diffuse light, but prevented pattern vision. Cotton gloves and cardboard cuffs extending beyond the fingertips restricted perception by touch. Their auditory perception was limited by a U-shaped foam rubber pillow on which their head lay and by a continuous hum of air conditioning equipment, which masked small sounds. Under the conditions of these experiments, cut off from stimulation, the content of thought patterns experienced a progressive change. Surprisingly enough, many of these individuals developed actual hallucinatory episodes.

Electroencephalographic tracings obtained during the course of these ex-

periments also showed progressive changes in the rhythms, primarily evidenced by slowing of the alpha peak.

The extent to which descending pathways may modify the activity of the reticular system, and in fact may even operate to the sensory ending cells, has been mentioned in a previous *Annual Review of Physiology* (7). The effect of cortical inhibition on the reticular system has also been demonstrated by Jouvett, Benoit & Courjon (8). These investigators studied the responses obtained from the auditory relay stations in the thalamus, from the reticular formation, and from the auditory cortex during auditory stimulation which was carried out simultaneously with stimulation of various regions of the cortex. They observed that stimulation of the cortex was capable of blocking the response at various of the relay nuclei. It has further been demonstrated by Galambos (9) that these inhibitory influences extend as far back as the end organ itself, and that modification of the cochlear response to standard tonal stimuli can be accomplished by brain stimulation.

THE ANATOMY AND FUNCTION OF THE RHINENCEPHALON

Anatomical and physiological experiments suggest that the rhinencephalic system may also play a part in the maintenance of consciousness. This possibility has been emphasized by Adey, Merrillees & Sunderland (10). These investigators observed behavioral changes and anatomical degenerative responses following removal of the entorhinal and other areas. Bilateral removal leads to docility, but there is preservation of the primary and slow wave responses of the hippocampus to stimulation. It is believed that these responses are mediated through the fornix from stimulation of the thalamus and septal area. They observed spindle bursts following the primary response of the auditory cortex to auditory stimulation following removal of entorhinal area, and feel that this may indicate some "waning of consciousness."

Similar studies are reported by Green & Adey (11). These investigators studied by electrophysiological techniques the afferent and efferent pathways from the hippocampus. It is their conclusion that the afferent fibers to the hippocampus project through the fornix with the dentate gyrus as an intermediary relay. Efferent connections are to the limbic cortex, the amygdala, the diencephalon, and mid-brain tegmentum. They suggest, that "if the amygdala represents the basal ganglia of the visceral system, perhaps the hippocampus corresponds to its cerebral cortex." The commissural and septal connections of the hippocampus in the rabbit have also been studied by Cragg & Hamlyn (12). Their studies indicate that ascending impulses from the intralaminar nuclei of the thalamus and septal regions are carried through the fornix to the hippocampus, thence to the entorhinal area. This area in turn projects through the external capsule, the anterior commissure, and the striae medullaris to the midbrain tegmentum, thus completing a recurrent pathway. The concept of the hippocampus as an associational area similar to the isocortex is disputed by Blackstad (13). This author has made an extensive anatomical study of degenerative changes demonstrated by Nauta's reduced silver method. It is his opinion,

that the findings made in this study may perhaps be taken as suggesting that hippocampus within the area dentata largely represents an effective relay mechanism for ipsilateral and commissural impulses far more than an integrated mechanism in the sense of, e.g., the associational areas of the isocortex.

Studies have also been carried out to determine the functional significance of the amygdaloid nuclei complex. Previous studies by Kaada, Andersen & Jansen (14) have suggested that the amygdaloid complex actually combines two separate functions. The phylogenetically younger basal lateral division, which appears to have no direct connections with fibers from the olfactory bulb, and whose efferent projections are unknown, appears to have functions closely correlated with those of the hippocampus, medial prefrontal, limbic, retrosplenial, and hippocampal gyral areas. The phylogenetically old antero-medial division receives fibers from the olfactory bulb and projects to the septal preoptic and hypothalamic areas. Stimulation of the former area has been thought to produce emotional responses, whereas from the latter area, controversial movements and licking, sniffing and other visceral responses have been noted. Machne & Segundo (15) recorded with a microelectrode the responses of single cells within the amygdaloid nucleus to stimulation in the tegmentum, olfactory nerve, vagus, sciatic, tooth pulp, auditory, visual, cutaneous touch stimulation, and pinching of the organs within the gastrointestinal tract. The most striking responses were obtained from stimulation of the sciatic nerve, somatic stimulation, and olfactory stimulation. In respect to these three modalities, marked convergence of influence in the single cells of the amygdaloid nucleus was demonstrated. The experiments were believed to support the concept that the amygdaloid nucleus serves as a correlation center for both olfactory and somatic afferent information.

Recent experiments have also been helpful in elucidating the conflicting reports of the results of ablations in this area. The effect of such lesions on experimentally neurotic animals has been reported by Masserman & Pechtel (16). The authors report certain neuropsychiatrically significant findings derived from six years of study on the effects of various cerebral lesions on the normal and experimentally neurotic behavior of 50 cats, and 40 monkeys. The animals were rendered "neurotic" through exposure to conflictful experiences. After these changes of behavior had become adequately stabilized over a period of 3 to 24 months, the animals were subjected to cerebral operations involving lesions in the thalamic nuclei (29) and the cingulate areas, the orbitofrontal areas, and the amygdaloid nuclei. Effects observed which seemed to be common to these lesions regardless of location were:

1. Moderate to extensive amnesia.
2. Amelioration or disorganization of neurotic patterns.
3. Diminished adaptability in behavior leading to (a) stimulus-bound perseveration and (b) impaired versatility and skill in problem-solving; and
4. Possibly as a result, "affective" changes characterized by a greater susceptibility to reactions indicative of startle and fear, with a corresponding increase in aggressivity.

Specific effects varied not only with the site and duration of the lesion, but with the species of animals and with the preoperative and postoperative

experience of the animal. For example, animals with great avidity for learning preoperatively showed less postoperative impairment as compared with less adaptable animals. Animals which had been made experimentally neurotic became much more markedly and diffusely hostile and destructive after mediodorsal thalamotomy than did nonneurotic animals used as controls. The changes in the four amygdaloidectomized male cats varied with time—they were friendly for about three months postoperatively, then became more aggressive again. It was also the impression of these observers that early retraining reduced the disability of the operated animals.

The authors emphasize the difficulty of carefully controlling such experiments; such variables as the unique genetic experience or background of each animal, subtle variations in handling, the difficulty of objective observation, and the inability to produce absolutely reproducible surgical lesions all constituted uncontrollable variables.

A further elucidation of the conflicting and changing results noted is presented by Weiskrantz (17). The author compared the results of lesions in the amygdaloid and medial-temporal regions, the inferior temporal convexity, and of control animals who were subjected to the operative procedure without cerebral lesion. The animals were observed for changes in their general behavior, but particularly in respect to alterations in a conditioned avoidance and a conditioned depression test. The conditioned avoidance reaction was determined through the use of a shuttle box, with an open door between two compartments. One compartment was lighted, the other unlighted. Every 30 sec., the lighting arrangement was reversed, and a shock was applied to the animal if he was in the dark compartment. The animal was conditioned to this box to the point where he would avoid the shock when the proper premonitory stimulus was presented. The rate of extinction of this conditioned response was also determined by following the same procedure, but with omission of the electric shock. The investigators discovered that following lesions in the amygdaloid nucleus, there was no severe loss either in the retention of the previously learned response or in the ability to acquire new avoidance learning. The striking feature of the experiments, however, was the demonstration that extinction of a previously learned conditioned response took place very rapidly in those animals which had been subjected to removal of the amygdaloid-medial temporal area. This was not observed in two other groups of animals. In addition, these animals showed tameness in response of their handling. "If man is at least partially a conditioned aversive stimulus to the monkey, then postoperative tameness may be the result of rapid extinction of avoidance responses to man." These facts suggest that particular aspects of postoperative treatment can be of great importance in producing tameness, on the one hand, if previous avoidance responses are permitted to extinguish, or increased fear on the other if additional aversive stimulation is introduced. The authors have observed that,

In a preliminary test of this general notion, which also has support from an analysis of the effects of aggressiveness of cage mates on operated animals, that the

amygdaloid-medial temporal animal treated gently and in a pet-like fashion after operation remained tame for several months, whereas one treated indiscriminately like a normal laboratory animal was grossly indistinguishable from a normal within a period of six weeks.

The authors also put forward a tentative suggestion that,

The effect of amygdectomy is . . . to make it difficult for reinforcing stimuli⁹ whether positive or negative, to become established or to be recognized as such. Sometimes the difficulty is seen in over-generalization, as when an animal attempts to ingest any object within reach, and sometimes it is in the lack of appropriate responses to any stimulus, as when an animal must be taught to eat.

It is not assumed that the behavioral alteration is a static one; immediate postoperative experience would be of great importance in establishing a particular repertoire of primary and secondary reinforcers.

Other studies have been carried forward to determine the emotional responses to stimulation of various areas, including the rhinencephalon. Further studies have been completed by Delgado, Rosvold & Looney (18). An assembly containing six stimulating electrodes was implanted in mesencephalic, diencephalic, and rhinencephalic structures in the brains of ten monkeys which had been trained to avoid shock of the feet. Stimulation of the medial nucleus of the amygdala and adjacent tissue in rhinal fissure, trigeminal nerve at the trigeminal ganglion, rostral part of the pons, medial part of the mesencephalon in the vicinity of the central grey, nucleus ventralis posteriomedialis, external part of the nucleus ventralis posterolateralis of the thalamus, and external medullary lamina of the pallidum produced a response similar to the conditioned fear response. Electrical stimulation of the sensory motor cortex, sensory motor pathways, nucleus ventralis lateralis of the thalamus, pulvinar of the thalamus, substantia nigra, part of the tegmentum inferior to the central grey, anterior hippocampus, posterior portions of the amygdaloid nucleus, and the putamen did not evoke the avoidance response. The authors felt that the electrical stimulation at least in the amygdala and the central grey induced in the animal a condition similar to that which is present when it is anxious or afraid of being hurt, and they cite evidence by Papez and MacLean to support the conclusion that emotional expression is mediated through the related cerebral structures called the limbic system. The responses obtained from the trigeminal nerve and other similar areas were considered to be simple responses to pain.

The authors point out one discrepancy in that stimulation of certain amygdaloid nuclei as well as the anterior hippocampus failed to produce a similar response.

In contradistinction to these results, the observations of Olds (19, 20) provide interesting evidence that the effects of activation of these areas are by no means entirely unpleasant. Rats were prepared with implanted electrodes in the areas to be studied, and were tested in a cage so devised as to make it possible for the animal to stimulate his own brain through pressing

a lever located within the box. It was discovered that with the electrodes located at certain areas, the animals would continue to stimulate themselves until such time as the current was turned off, after which they would make a few more trials, then usually give up and go to sleep. Experiments were then devised in which the desire to stimulate the brain could be made to compete with the desire for food. It was possible to demonstrate that under these circumstances the desire for stimulation of the brain was greater than the desire to eat, even in the starved animal.

Using this technique, quantitative data have been obtained as to the degree of motivation for self-stimulation observed with various locations of the stimulating electrodes. When electrodes were implanted in the classical sensory and motor systems, response rates stayed at the chance level of 10 to 25 an hour. In most parts of the midline system, the response rates rose to levels of 200 to 5000 an hour, definitely indicative of a rewarding effect of the electric stimulus. But in some of the lower parts of the midline system, there was an opposite effect: the animal would press the lever once, and never go back. This indicated a punishing effect in those areas (these are areas observed by Delgado, Roberts and Miller to produce an avoidance effect, and where Hess and others have found responses of rage and escape). The greatest reward seems to come from stimulation of areas in the hypothalamus and certain midbrain nuclei, presumed to be centers for the control of digestive, sexual, excretory, and similar processes. In the rhinencephalon, the effects were milder, producing self-stimulation at rates of about 200 times an hour.

Efforts are now being made to determine whether these "drives" can be modified. It has been demonstrated that the hungry animal will show greater "appetite" for electrical stimulation in some brain regions. Similarly, there are areas where the rewarding effects of a brain stimulus can be abolished by castration and restored by injections of testosterone.

Our present tentative conclusion is that emotional and motivational mechanisms can indeed be localized in the brain; that certain portions of the brain are sensitive to each of the basic drives. Strong electrical stimulation of these areas seems to be even more satisfying than the usual rewards of food, etc. This finding contradicts the long held theory that strong excitation in the brain means punishment. In some areas of the brain it means reward.

LOCALIZATION OF FUNCTION IN NEOCORTEX

Audition.—Increasing attention is being paid to the potentialities of electrophysiology for the understanding of the mechanism of hearing. An interesting review of new developments in this field is presented by Rosenblith (21). The recording of the cortical response to auditory stimulation has proven useful in several applications. Derbyshire, *et al.* (22) report clinical use of this technique for audiometry. A similar technique is also proving useful for the study of the localization of tone function in the cochlea. A study of this sort has been reported by Kimura, Schuknect, & Hutton (23). The authors describe a recording technique for determining auditory thresholds in animals. The threshold is determined by recording the cortical response in the

animal anesthetized with pentobarbital. Strychnine sulfate (3 per cent) was applied to the auditory cortex to facilitate the response to sensory stimulation of the ear. The authors used the "least visible" response from the strychninized cortex as the definition of a threshold response, and defined this arbitrarily as that intensity which yielded approximately 75 per cent responses. Using this technique, the authors determined the effects of microscopic lesions in the cochlea. Their findings support the previous concepts of an orderly frequency distribution within the cochlea, high frequencies at the basal and low frequencies at the apex.

Ablation experiments are further expanding the recognition of the remarkably wide representation of auditory function in the cortex. Neff, and his co-workers (24) have made a series of experiments on the effects of cortical ablations on various functions related to hearing. Bilateral ablations in cats of area A_1 and in some instances A_2 and E_p produced severe and permanent defect in the ability to localize the source of origin of a sound. The cats were trained to respond in a test situation according to the location of an auditory stimulus. This performance was lost after the ablations noted. That these losses were specific was demonstrated by the fact that other learned habits were retained, and the animals continued to retain their ability to respond to proper visual stimuli.

In subsequent experiments Butler, Diamond, & Neff (25) showed that similar extensive ablation did not destroy the ability of the animals to discriminate changes in the frequency of a sound. There occurred a temporary amnesia for a learned habit, but all animals were able to relearn to respond to different auditory frequencies. The areas removed comprised those in which an evoked auditory response has been demonstrated, but does not include all areas known to receive projections from the medial geniculate body. These observations have also been confirmed by Sharpes & Jasper (4). Erulkar, Rose & Davis (26) have reported the results of extensive studies of micro-electrode recording from the auditory cortex of the cat. The animals were stimulated with clicks and with sounds of various frequencies. Under light general anesthesia, 34 per cent of all units isolated were unresponsive to sound stimuli. The majority of units responded insecurely and only about 14 per cent of all units responded each time to an adequate stimulus. Units responsive to sound can be encountered in all cellular layers of the first auditory field.

It was observed that a total stimulus may either suppress discharges of spontaneously active units, or it may excite a unit to discharge. A unit may respond only at the onset of the tone, or it may respond for greatly varying periods of time during tonal presentation. Units sensitive to tones are as a rule optimally sensitive to restricted frequency bands. The evidence at hand implies that a cortical locus is probably not uniquely critical for the optimal frequency response of a unit. Nevertheless, within the posterior segment of the first auditory field, most units studied were optimally sensitive to low frequencies. The authors were able to demonstrate masking and similar phenomenon well recognized in ordinary auditory testing.

Speech.—It was pointed out in last year's review that recent evidence has suggested that a close correlation between handedness and cerebral dominance for speech does not exist. A recent review of this problem is that by Ettlinger *et al.* (27). According to their analysis, recent reports have clearly indicated that it can no longer be accepted that right cerebral dominance is the rule in left-handed individuals or that aphasia resulting from a left-sided lesion in a left-handed patient is in any way exceptional. Analysis of reported cases would even suggest that "left-brainedness" might well appear to be the more prevalent form of cerebral organization in left-handed individuals. The authors review ten of their own cases of cerebral lesions in left-handed individuals in an effort to determine the nature of the speech disturbances observed. Their major effort is to determine whether in the left-handed individuals a firm left-sided dominance can be demonstrated or whether, in the sinistral, speech is ordinarily represented bilaterally—a view first advanced by Chesher (28).

In the 10 cases of exclusively or predominantly unilateral brain disease in left-handed individuals reported in this review, the lesion was left-sided in eight cases and right-sided in two. These cases were then analyzed on the basis of the location of the lesion, and the presence or absence of dysphasia, dysgraphia, dysparaxia, and the Gerstmann syndrome. On the basis of this analysis it is concluded that, of these cases, the presumptive hemisphere dominance was clearly on the left in five cases, left or indeterminant in two cases, right or indeterminant in two cases, and right for language and left for praxis and topognosis in one case. It is concluded that where some degree of cerebral ambilaterality may exist in a certain proportion of cases, unilateral representation of speech (usually left, but occasionally right) is the most prevalent form of cerebral organization in sinistrals.

These findings are strongly in agreement with the much larger series of cases reported by Roberts (29). This author presented the results of cortical excision. Because of the fact that with infantile injury of left hemisphere of the brain, the right hemisphere may assume the major cerebral functions, individuals suffering from hemiplegia in early life were excluded from

TABLE I
RELATION OF HANDEDNESS TO APHASIA FOLLOWING
UNILATERAL CEREBRAL LESIONS

	Lesion on Left	Lesion on Right
<i>Right Handed</i>		
Total	157	196
Aphasic	115	1
<i>Left Handed</i>		
Total	18	15
Aphasic	13	1

Roberts' series. Under these circumstances 73.2 per cent (115 of 157) of his right-handed individuals as compared to 72.3 per cent (13 of 18) of the left-handed had aphasia after operation on the left hemisphere. Similarly 0.5 per cent (one of 196) of the right-handed as compared to 6.7 per cent (one of 15) of the left-handed patients had aphasia after operation on the right hemisphere. These findings indicate that the left hemisphere is the dominant one for speech regardless of handedness.

This paper also contains interesting data regarding the possibility that following a lesion in the dominant hemisphere the nondominant hemisphere may assume language functions. 24 of the patients included in this series were known to have aphasia as a result of an original injury which occurred after the age of two years. Two-thirds of these patients again had aphasia after an excision in the dominant hemisphere. However, aphasia occurred less frequently in these cases with previous gross injury of the brain (60 per cent) than did in those who had had no previous acute gross trauma to the left hemisphere (80 per cent).

Hecan & Purcy (30) have made an interesting study of the aphasic defects and disturbances observed in individuals suffering from focal epileptogenic disorders involving speech. Going through the records of 3,000 cases, they found 126 who were known to have clearly demonstrated focal epileptogenic lesions, and who described an aura in association with these seizures; of these 97 were right handed and 29 were either left handed or ambidextrous. From analysis of these records, from a review of the literature and from analysis of 12 of their left-handed individuals with permanent right hemisphere lesions (as opposed to the temporary "paroxysmal" disturbances noted in the epileptic patients), they reach the following conclusions. Dysphasia as the aura of a focal epileptic seizure is more common in left-handed individuals than it is in right-handed individuals regardless of the side on which the epileptogenic focus is located (Table II). It will be noted that among the left-handed individuals, 17 out of the 18 cases having a left-sided focus were dysphasic. In those instances where the focus was on the right,

TABLE II
RELATION OF HANDEDNESS TO LOCATION OF EPILEPTOGENIC FOCUS
IN PATIENTS WITH AURA OF APHASIA

	Focus on Left	Focus on Right
<i>Right-Handed</i>		
Total with aura	63	34
With aura of aphasia	31	4
<i>Left-Handed</i>		
Total with aura	18	11
With aura of aphasia	17	9

nine out of a total of 11 cases had this aura. In the case of the right handed individuals, however, an aura of dysphasia occurred in only four of 34 cases having the focus on the right.

The second observation noted is that regardless of handedness, individuals with a lesion in the left hemisphere were more likely to have an aura of some sort than was the case when the lesion was in the right hemisphere. Thus, of 223 patients with a left sided lesion, 81 experienced an aura, whereas of 191 patients with a right sided lesion, only 45 noted an aura.

In reviewing patients with permanent destructive lesions of the brain, they found only one out of 12 left-handed individuals who suffered from permanent aphasia. The authors state,

It is probably not an exaggeration to say that the evidence concerning permanent dysphasia favors the suggestion that a left-handed patient with a unilateral lesion of either hemisphere has a better chance of escaping a dysphasia or of recovering from it more rapidly, than a right-handed patient with a left hemisphere lesion.

The authors suggest that the frequency of involvement of language in paroxysmal disturbances of either hemisphere in left-handed people, with the apparently paradoxical observation that permanent dysphasia is less common in left-handed individuals suggests that, in the left-handed individual, the language function is more widely distributed and presumably has a greater bilateral representation than is the case in the right handed individual. They conclude,

We already have some evidence to suggest that language representation is more equally distributed between the two hemispheres in left-handed than in right-handed people. A possibility that should not be overlooked is that the bilateral representation of language in left-handers is only one aspect of the increased diffusion of language organization in the subjects. That is to say, it is conceivable that in left-handers, the mechanism of speech involves a greater area of cerebral tissue within a single hemisphere than is the case with right-handers. . . . We are in effect postulating a greater degree of equipotentiality in the cerebral mechanisms for language in left handed and ambidextrous people than in right-handed people. But in view of our results, this supposition involves us in the further assumption that greater equipotentiality is likely to involve greater sensitivity to acute interferences of relevant function.

These observations are of particular interest in relation to the pioneer observations of Orton (31) that language disturbances of children are more common in left-handed and ambidextrous children than in clearly right handed individuals. [For critical re-evaluation of this thesis, see Hallgren (32) and also Drew (33).]

It should be noted that the observations of Hecaen & Purcy regarding the infrequency of permanent dysphasia in left-handed individuals, which they developed from a rather small series of personal cases, is at variance with several other more extensive reports in the literature. Thus, Goodglass & Quadfasel (34) present 13 personal cases plus 110 cases from the literature (Table III). These figures indicate that approximately 80 per cent of left-handed individuals develop aphasia after a lesion regardless of whether this

TABLE III

RELATION OF APHASIA TO UNILATERAL CEREBRAL LESIONS

	Lesions of Left	Lesions of Right
Total Cases	61	62
Number with Aphasia	53	50

lesion is in the right hemisphere or in the left hemisphere. To that extent that their data are a fair sample of left-handed patients, with appropriately situated unilateral lesions, it can be concluded that many left-handed people have bilateral representation of language, and that they may develop aphasia regardless of which hemisphere is affected.

In consideration of the above reports, it is clear that there is no close correlation between handedness and the laterality of the language function—that in general, the left hemisphere is dominant for language regardless of the handedness. Hecaen's data present considerable evidence to suggest that in the left-handed individual both hemispheres are more likely to be involved in the language function, but this suggestion has not been entirely supported from other data, especially in respect to his conclusion that the left-handed individual is less susceptible to permanent dysphasic symptoms following a destructive permanent lesion. A possible explanation for this apparent discrepancy may lie in the definition of "permanent dysphasic symptoms." It may well be that the left-handed individual, with wider cerebral representation of language functions, is more susceptible to minor transitory disturbances, but less susceptible to severe permanent loss of speech.

Two other approaches to the problem of language disabilities might be mentioned. Webb & Lawson (35) report an electroencephalographic study of children with severe language disabilities. From 700 children suffering from severe language disabilities, they selected 41 patients, who were considered to have no evidence of organic neurological impairment, emotional disability, or intellectual retardation. Of these 41 cases, 24 were observed to have abnormalities of EEG. In 11, these abnormalities were focal in character. The authors consider that these findings suggest that an organic cerebral defect may be present in a considerable number of children with speech and reading disabilities, even though no clinical evidences of such defects are noted.

A study of a familial form of word blindness has been made by Drew (33). The author reviews the literature relative to familial congenital word blindness and indicates that "the genetic statistical analysis shows that specific dyslexia with a high degree of probability follows a monohybrid autosomal dominant mode of inheritance." Thus, a single gene is involved in a dominant nonsex linked form of inheritance. In view of this observation, the author considers it inconsistent that such an inherited trait could exist in the absence of other associated neurological abnormalities. Such an eventuality would require a return to the now generally outmoded concept of discrete cerebral

localization of function. The author then presents the results of careful study of three individuals suffering from this disorder.

The investigations demonstrate that there is evidence that something more than a pure language disturbance exists in the patients studied. There was observed a tendency to confuse yellow and white, and to rotate blocks in the block design test, changes which are difficult to ascribe to a language disturbance. In addition, there was an abnormal response in a face-hand test, and right-left disorientation, again findings not easily viewed as manifestations of aphasia. Evidently, although these patients show evidence of aphasic language disturbance, they also appear to suffer from agnostic difficulties as well.

The author then refers to previous studies with nonfamilial cases of congenital dyslexia, and reports that without exception patients studied by Rabinowitch and others have shown one or more of the defects detected in the cases studied here. Thus, in addition to the reading disability, a defect in directional selection, mixed hand-eye preference, face-hand test abnormalities, reversals, auditory-visual phonetic disintegration, and spatial disorientation are found in varying combination.

In the light of these observations, word blindness and dyslexia are both considered by the author to represent inadequacies in Gestalt function, with interference with visual-verbal comprehension. It is the opinion of the author that not only reading, but writing, spatial orientation, and auditory-visual integration may be affected by this defect which is perhaps the result of a failure of or delay in maturation of the function of the parietal lobes. If viewed as defects in Gestalt function, many of the reported correlates of reading disability become comprehensible as parts of a single fundamental defect—a defect in correct figure-ground recognition in both familial and non-familial congenital dyslexia. The frequently reported absence of neurological findings in congenital word blindness is not borne out by the present investigation. Very similar conclusions are also presented by Hermann (36).

The complexity of the problem of language disabilities continues to be emphasized by clinical and pathological studies. Single case reports by Klein & Harper (37) and Riese (38) indicate the inadequacy of pathological studies, in which the lesions are likely to be disseminated. It is certain that the total mass of tissue destroyed is of crucial importance in the severity and persistence of the aphasic disability. The importance of complex intellectual functions is also emphasized by the study of Birch (39). This observer noted that many patients with expressive aphasia still have ejaculatory or fragmentary speech patterns. It is his thesis that part of the problem in these individuals may result from inhibition of speech function through the destruction of certain areas which exert a controlling influence on speech. He attempted to test this thesis by introducing a stimulus to mask out or reduce the activity of the auditory receptive areas—assuming that these areas are actually inhibiting the motor function. He therefore attempted to retrain individuals for speech under conditions of loud noise. Under these conditions, 75 per cent of the individuals tested improved in ability at name finding or reading out

loud. He observed that this improvement persisted for a period of time after the end of the test situation. Similar findings have also been reported by Cherry, Sayers & Marland (40).

Spatial orientation.—Considerable interest has centered around the question of the significance and nature of disturbances resulting from lesions of the nondominant hemisphere. Several papers have been presented which explore the hypothesis that lesions of the nondominant hemisphere may produce specific disability in spatial relationships of a form not observed with lesions in the dominant hemisphere. Heilbrun (41) made a study for the purpose of testing the hypothesis that subjects with lesions of the left hemisphere showed the greatest performance decrement on tasks involving verbal knowledge or skills, while those with right sided lesions showed a marked disability on tasks involving spatial or spatial-temporal relationships. The objective of the study was to determine whether right-handed individuals with left hemisphere cerebral lesions and those with lesions of the right cerebral hemisphere would perform differentially on either of two types of psychological tests. In order to test this hypothesis, a series of performance tests were administered, some of which required verbal responses and some of which depended upon a knowledge of spatial-temporal relationships.

On the basis of these tests, it was demonstrated that the left hemisphere groups showed a significant inferiority to the group with right-sided lesions on the verbal measures. However, this difference was not present when the subjects with dysphasic symptoms were excluded from the left hemisphere group.

There was no difference between the left and the right hemisphere groups with respect to spatial testing performance. The studies of this group thus did not substantiate previously reported observations, these findings tending to suggest that patients with lesions of the right hemisphere show a greater disturbance of spatial-temporal relationships than do similar patients with lesions of the left hemisphere.

These findings are not entirely supported by those of Hecaen *et al.* (42). The authors report the findings on postoperative examination of 16 patients subjected to removal of tissue from the temporal-parietal-occipital junction of the nondominant hemisphere. In 14 of these patients the excision was from the right hemisphere of a right-handed individual. In two instances the excision was from the left hemisphere of a left-handed individual, and in these instances, the typical syndrome did not occur. Nine of the reported cases exhibited the typical picture. The defects produced by these lesions are primarily defects of perception and of interpretation. The apractognosic syndrome resulting from lesions in the right cerebral hemisphere includes a group of manifestations related to alterations of somatognosia. In addition to body-scheme disturbances of the anosognic type, there are visual constructive disabilities, apraxia for dressing, difficulties in spatial orientation (unilateral spatial agnosia, disturbances of topographical relations), and alteration of visual coordinates. Loss of topographical memory is also a probable constituent of the syndrome. The anatomical lesion producing these

symptoms includes as a common ground the supramarginal gyrus, part of the angular gyrus, and the posterior part of the post-temporal convolution. Lesions which did not include this area did not produce the typical picture. In general, the severity of the disability was increased with involvement of more widespread areas. In general, the severity of the lesion also increases with the increasing age of the patient, and the authors are careful to point out that they are not implying a fixed relationship between anatomical structure and symptom. They remark,

Even though we recognize the importance of the somatognosic disturbances as the central element of the syndrome of apractognosia, we are not attempting to assign such a function to a definite structure. We do not even feel that a somatognosic function should be thought of as an isolated entity, any more than the "sense of space" or the "function of visualization." The individual develops such capacities by the constant integration and utilization of many functional areas. The peculiarity of the training and experience of one person, as compared with those of another, might well produce individual variation in the functional organization in this area of the brain. In addition to the localization of lesions which cause disturbances of the perception of space relationships or body scheme, one should consider how the patient with this lesion is experiencing his defect exteriorizing it or compensating for it, and by what means he is doing so.

The authors point out interesting distinctions between the disturbances produced by a right-sided lesion, and that which results from the corresponding lesion on the left. The disturbances produced by the right-sided lesion are almost exclusively left-sided. In the case of a lesion in the dominant hemisphere, however, bilateral disturbances are observed, and the quality of the disturbances is different. The individual with a right-sided lesion has forgotten his body half or denies his hemiplegia and behaves as if his defect were absent. The patient with the left-sided lesion, experiencing finger agnosia, or autotopagnosia, is aware of his body, but simply does not grasp the relationship of the position of its different parts. It is suggested that this important distinction may be closely related to the fact that language functions appear to center in the left hemisphere. The language functions being bilateral, and almost certainly intimately related to body scheme, are therefore associated with at least some degree of bilateral representation of somatognosia. Therefore, the part of the dominant hemisphere subserving somatognosia must surely have close connection with the speech areas. If a lesion is produced in a major hemisphere, the connection between body scheme and language will be dissociated once more in varying degrees; but traces of the connections persist and continue to modify the effects of dominant-hemisphere lesions so that the picture presented is different from that of the minor.

Another report suggesting a rather specific defect associated with lesions in the nondominant hemisphere is by Fisher (43). This observer noted that lesions of the right hemisphere produced an inability to carry through a motor act, for example, the individual may be unable to keep his eyes closed or deviated in a given direction, to protrude the tongue or keep the mouth

open. The hand grip is brief. There is a tendency for the patient to "peek" during sensory examination. Most of the individuals exhibiting this phenomenon experienced impairment of lateral gaze, and the observer considered it to be a symptom of a frontal lobe lesion.

An interesting observation which may be relevant to the ability of an individual to transfer learning from one hemisphere to the other is that of Meyers (44). When the crossed optic fibers are destroyed in the cat by midline section of the optic chiasma, each eye then discharges into only the ipsilateral hemisphere over the surviving uncrossed fibers. Cats so prepared are able to perform with one eye simple pattern discriminations learned with the other eye. This interocular transfer in the absence of the crossed optic fibers requires a neural interaction across the midline. When, in such animals, the corpus callosum is now destroyed, this interocular transfer is lost. When such animals are trained to perform pattern discriminations with a mask covering one eye and a consistently high level of performance has been obtained, when the mask is shifted to the other previously unmasked eye, performance drops abruptly to a chance level.

It is evident that when a certain function has been learned by one hemisphere, the corpus callosum is capable of determining the transfer of this function to the other hemisphere, and when the corpus callosum has been sectioned, this capability is lost.

CONDITIONING AND THE EEG

The development of new techniques for depth recording, and the ability to use these techniques in the unanesthetized animal has made possible an expansion of knowledge of the neuronal activity associated with the conditioning process. The fact that changes in the electroencephalogram could be produced by a conditioned stimulus has been recognized for many years. Newer techniques, however, are permitting increasing refinement of our knowledge of the nature of the responses, and the structures involved in various types of conditioned responses.

In regard to the areas of the brain which may be involved, it has been demonstrated that the occipital rhythm is by no means the only electroencephalographic feature which may be modified through a conditioned stimulus. Gastaut *et al.* (45) have demonstrated that, if a sensory stimulus is used as the unconditioned stimulus and a sound as the conditioned stimulus, it is possible to observe flattening of the electroencephalographic tracing of the contralateral rolandic rhythm (rhythm *en arceau*) in response to the sound without the sensory stimulus. It is possible to get blocking of the rhythm in response to a sound without the necessity for a somesthetic stimulus. The conditioned rolandic blocking is also obtained when the passive proprioceptive stimulus is replaced by an active proprioceptive stimulus such as occurs when the subject makes a fist on command. This response can be further conditioned by instructing the subject to make a fist in response to a light stimulus—the auditory stimulus being delivered prior to the light stimulus. Under these conditions, the light stimulus does not produce its

usual blocking of the occipital alpha, but rather serves to fortify blocking in the rolandic area. The authors thus demonstrate that, through modifications of the interrelationships of various stimuli, alterations in cortical excitability can be produced, and the electrical responses from stimuli significantly modified from their usual pattern. They conclude,

Simultaneous electroencephalographic studies of different territories, therefore, discloses in man the dynamic processes taking place in the cortex. This method allows to give objective evidence of the elaboration of temporary connections and of the mobility of foci of cortical excitation and inhibition.

It has been demonstrated that a conditioned response can be obtained through direct electrical stimulation of the motor area of the cortex by Doty, Rutledge & Larsen (46). These authors demonstrated that it is possible to produce a movement of an extremity when electrical stimulation of the cortex is used as the unconditioned stimulus. In order to be certain that this response was not actually a conditioned response from some sensation of the skin or meninges produced by direct electrical cortical stimulation, the authors carried out bilateral fifth nerve sections and excision of the meninges without altering the response. Cortical excision, however, eliminated the conditioned response.

Modifications of the conditioned reflex, and especially the role played in them by the activating systems of the thalamus and midbrain have been the subject of several important studies. Morrell & Jasper (47) describe a technique which makes it possible to record the conditioned cortical electrical responses in normal unanesthetized monkeys. Using subgaleal or extradural leads, these authors recorded the electroencephalograph of unanesthetized monkeys using a flashing light as the unconditioned stimulus, and tactile auditory or visual stimuli as the conditioned stimulus. Two distinct forms of conditioned responses were obtained. The first was a repetitive discharge (photic driving) at the flicker frequency. The second, and more stable response, was a well localized desynchronization or "activation pattern." Both of these responses were limited to the occipital and paraoccipital areas. A definite sequence of events preceded the ultimate establishment of these two forms of conditioned response.

Before conditioning begins, the unconditioned stimulus evokes a "photic driving" response from the posterior head regions. The conditioned stimulus (tone, touch, or light) at first leads to a general alerting response with external evidences of alerting, and EEG "desynchronization" or "activation." All components of this response show adaptation, and diminish or finally disappear with repeated presentation of the stimulus. Any alteration in the stimulus or in the experimental situation restores its activity.

This regional "alerting" response eventually disappears, but with the initiation of paired trials, during which the conditioned stimulus is paired with the unconditioned stimulus, the "alerting" response reappears because of this alteration in the test situation. As paired trials are continued, the

diffuse activation pattern gives way to a precise discharge specific to the flicker frequency and limited to the occipital regions. Finally, with the recording techniques used, the repetitive discharge is replaced by focal low voltage, fast activity which is also sharply limited to the occipital area.

These findings suggest that alpha blocking or desynchronization corresponds to a definite functional state of the cortical area involved, and activation or excitation of the neuronal units therein.

The authors observe that, in the final states of the conditioned reflex, there remains localized desynchronization in the occipital region—that is, the area in which the conditioned response is activated. Two possible interpretations of this phenomenon are suggested. One is that this represents a portion of the orienting reflex, or generalized activation which persists and remains within the conditioned reflex as a requisite for the adequate perception of the signal stimulus. The other explanation may be that it represents a nonspecific sign of cortical activation, the result of a variety of unrelated neural events. The localized blocking may be a new positive conditioned activation entirely separate from the orienting response.

Previous studies of the brain stem reticular system and the thalamus have indicated that there may be two alerting mechanisms, one a generalized one, and the other a more local and specific activation. It is thus probable that the eventual localized blocking of alpha activity represents local facilitation or "alerting" of this cortical area without the involvement of the entire cortex, and attributable to a specific thalamic mechanism. The transitory localized occipital frequency-specific repetitive discharge which, following paired trials, is elicited by a previously ineffective stimulus and is subject to differentiation, is regarded as an objective trace in cortical activity of a conditioned temporary connection.

Rather similar studies to those of Morrell & Jasper are reported by Gastaut *et al.* (48). The authors report an electroencephalographic study of conditioning in man. They tested 60 subjects. Observations were made of the changes in basic electroencephalographic rhythms in response to various combinations of visual, sensory, auditory, and kinesthetic stimuli. It was observed that the electroencephalographic responses could be conditioned, and that the nature and location of the electroencephalographic change varied in accordance with the modality of the conditioning and direct stimulus and its response.

From a consideration of the site and time relationships which characterize central excitation and inhibition, the authors distinguish four types of process: (a) the process of excitation which is generalized and not conditioned, coming on each time the brain receives a signal qualitatively or quantitatively new, this process evoking the unconditioned responses of surprise and attention with all their electropsychosomatic components; (b) the process of excitation which is localized and conditioned, arising whenever the brain receives a signal which is known and has acquired special significance because previously combined with other signals of ontogenic or his-

torical importance, this process being responsible for the acquisition of conditioned responses which govern adaptation and learning; (c) the process of inhibition which is localized and conditioned and comes on whenever a known signal loses its conditioned significance, a process which also plays a basic part in adaptation, since it causes suppression of conditioned responses which have become useless or harmful; (d) a process of inhibition which is generalized and not conditioned and appears in two different circumstances, (i) whenever the brain has received a new signal producing a response of surprise or attention, and (ii) whenever a new or known signal is repeated. The authors' study included an analysis of the neurophysiological interpretations of the conditioned EEG responses observed in these experiments. They separate the electrical phenomenon observed according to whether they precede, accompany, or follow conditioning.

Before conditioning, they observe a generalized cortical "blocking" of rhythms, which results from the application of any new stimulus. This they attribute to generalized cortical desynchronization resulting from activation of the brain stem reticular system as conceived by Moruzzi and Magoun.

In response to a stimulus which is closely and often repeated, they observed localized "blocking" of cortical activity, and this they attribute to activation of the thalamic reticular formation, a structure activation of which was shown by Jasper to put into play localized desynchronization of the appropriate overlying cortical areas.

The authors suggest that, when a stimulus is first applied, it is relayed to both of these reticular areas, but the lower brain stem area is more rapidly inhibited during adaptation to repetitive stimulation, and the effects of the more specific area become increasingly prominent during repetitive stimulation.

During the process of conditioning, a signal which has previously become ineffective as a result of habituation, when combined during conditioning with an effective signal, produces blocking of that region in which the effective signal is normally analyzed. It follows that the normally ineffective signal, from the moment it becomes conditioned, no longer travels along its usual thalamocortical pathways, but instead borrows those which had been reserved for the transmission of the effective signal.

After conditioning, the response can be inhibited in two ways. It can be inhibited by conditioned inhibition—which corresponds to inhibition of extinction, differentiation, and delay, and which depends on a temporary depression of the thalamocortical projection system, previously activated by the conditioned stimulus.

It can be inhibited by an unconditioned inhibition, corresponding to external or super maximal inhibition, and depending upon temporary depression of the brainstem reticular system.

Further studies by Dykman & Shurrager (49) lend further evidence to the widespread and fundamental nature of the conditioned type of response. Dykman & Shurrager developed a technique for the establishment of condi-

tioned reflexes in kittens previously subjected to spinal cord transection. The unconditioned stimulus was electric shock applied to the foot, and the responses to be conditioned was the movement of the foot. The conditioned stimulus was a light tactile cutaneous stimulus on either leg or on the tail. The authors demonstrated that movement of the foot could be conditioned to such tactile stimuli in both acute and chronic spinal animals. The period of conditioning and extinction was found to be a function of the number of trials. Within the time limit of the experiment, spinal conditioned response persistence and spontaneous recovery were not related to rest periods. The spinal conditioned response was not felt to be an artefact resulting from lowered skin resistance. The latency of the spinal condition response was greater and more variable than the latency of the reflex produced by direct electrical stimulation.

It is interesting that, not only can the conditioned reflex response be correlated with normal electrographic changes of cortical activity, but also with the type of pathological discharge observed in patients with convulsive disorders. An interesting interrelationship has now been demonstrated between the presence of a focal epileptogenic lesion of the cortex, and modification of the conditioned response which may result. As previously reported in this review by Jasper, Gloor & Milner (47), the presence of an actively discharging epileptic focus in the cerebral cortex has a serious inhibitory effect on the ability to produce a conditioned reflex involving the area of cortex so involved. Morrell, Roberts & Jasper (50), as described in a further report of this study, created epileptic foci in monkeys by the placement of alumina cream gel in various areas, including the primary sensory cortex, the auditory cortex, the postcentral leg area, the occipital lobe, and the amygdaloid-hippocampal region. With the development of an active lesion, the animals became incapable of developing a conditioned reflex. This loss was a specific one—lesions in the sensory area were followed by inability to respond to a conditioned sensory stimulus, but these animals could be conditioned with auditory or visual stimuli. However, in the case of animals with a lesion in the amygdaloid-hippocampal region, a generalized impairment of conditioned responses was observed. Excision of the epileptogenic foci was followed by improvement and restoration of the lost abilities.

It is evident that the formation of the normal conditioned response is dependent upon the proper functional state of the area of cortex involved. Data are not yet available as to the degree of flexibility or adaptability of the central nervous system to irritative or destructive lesions. It is certain, however, as shown by Berlin & Wolff (51), that interference with conditioning is an important aspect of brain damage if the lesion is of sufficient size. These workers tested 50 normal and 23 individuals suffering from cerebral lesions. Of these, 11 had focal areas of tissue destruction. They attempted to obtain a stable conditioned response to tone, a negative conditioned response to light, and calibrated tones, and a negative and positive conditioned response in sequence. Definite impairment was observed in the subjects with cerebral

damage, particularly in respect to their ability to relate the symbol to the significant subsequent event. Regardless of the site of the lesion, difficulties in the establishment of positive and negative conditioning responses were the same, and probably depended primarily on the mass of tissue lost, the complexity of the adaptive functions, and the rate at which tissue damage had been experienced.

From the paper of Berlin & Wolff, one derives the feeling that the specific location of the lesion is far less important for this type of "learning" than is the total mass of tissue involved, and that one must assume a considerable degree of adaptability and modification of function within the central nervous system. The extreme opposite view is that reported by Sperry (52). This investigator has been observing the effects of regeneration in the optic nerve of the newt. In this animal, effective regeneration of the nerve may occur, even when the eye has been removed and restored in an abnormal position. It has thus been possible to permit regeneration of the optic nerve into an eye rotated through 180° . Similarly, it has been found possible to rotate the eye through 180° without sectioning the nerve, the animal thus being provided with an "upside down" vision. Under these circumstances, the animal continues to respond in an abnormal fashion to visual stimulation, even though the condition exists for a period of as long as two years. The author concludes

Experiments cited here show that in the lower vertebrates, at least, many features of visual perception—the sense of direction and location in space, the organization of patterns, the sense of position of the visual field as a whole, the perception of motion, and the like—are built into the organism and do not have to be learned. More general experiments suggest that the organization of pathways and associations in the central nervous system must be ascribed for the most part to inherent developmental patterning, not to experience. Of the thousands of circuit connections in the brain that have been demonstrated, not one can demonstrably be attributed to learning. Whatever the neural changes induced in the brain by experience, they are extremely inconspicuous. In the higher animals, they are probably located in the more remote byways of the cerebral cortex. In any case, they are superimposed upon an already elaborate, innate organization.

There are probably few who would agree with this extreme view of the limitations for "learning" within the central nervous system, especially in view of the remarkable degree of adaptability and flexibility of electrical and physiological responses demonstrated in the above outlined studies of the conditioned reflexes.

In addition to the fact that the presence of an epileptogenic focus may interfere with the establishment of a conditioned reflex, a reverse effect has also been demonstrated, namely that under certain circumstances, the abnormal or convulsive type of discharge can be modified by a conditioned stimulus. Naquet & Morrell (53) produced a photomyoclonic epileptic response in cats treated with pentylenetetrazol (Metrazol). In these animals, the application of a photic flash stimulation regularly produced an electro-

encephalographic response and a myoclonic seizure. They then attempted to condition this response through an auditory stimulus. The results of these experiments were inconstant. It was their feeling that, in some instances, a photomyoclonic type of response could be conditioned to an auditory stimulus, but because of variability in the threshold and the occurrence of spontaneous responses in the pentylenetetrazol-treated animals, the validity of their results was uncertain. However, it was observed that in animals which had an epileptogenic focus in the occipital area produced by previous treatment with alumina cream, a conditioned response of this sort could regularly be produced.

A similar type of experiment in humans has been reported by Gastaut *et al.* (54). These authors attempted to condition the spike and wave and multiple spike and wave discharges unconditionally precipitated by intermittent photic stimulation in photosensitive epileptics, using sound as the conditioned stimulus. In two patients with a photosensitive form of *petit mal* attacks, in which the absences and myoclonic jerks were favored or precipitated by certain traumatic situations, conditioning was obtained after 20 trials of combined sound and light stimuli.

The authors express the opinion that reflex conditioned attacks are very frequent among epileptics, but are often not recognized as such because of the variety of stimuli which may constitute temporary links in a chain of conditioned reflexes and through which the actual relationship between stimulus and response may eventually become inapparent.

MENTAL IMAGERY, PERSONALITY, AND THE EEG

Studies of conditioning outlined above have clearly indicated that, during periods of activity, the rhythms of the active area of the cortex are reduced in amplitude and synchrony. Conversely, active inhibition of the cortex is likely to be associated with persistence of the rhythm, or even by its augmentation. Several studies have been carried out recently which indicate that there is a correlation between the mental characteristics of the individual, and the modification of rhythmic activity of the cortex which occurs during intellectual activities. There is also some suggestion that the resting pattern of cortical activity may also be correlated with the intellectual capacities of the individual.

Walter & Yeager (55) have correlated the ability of normal and blind individuals to form conscious images with changes in the electroencephalogram which occurred when visual imagery was consciously attempted. Studies were carried out as follows. In tests with normal individuals, the subjects were first permitted to open their eyes during an EEG recording, and they were shown certain standard diagrams. Subsequently, and with the eyes closed, they were asked to recall the picture of these diagrams visually. Finally, at the termination of the electroencephalogram, they were requested to reproduce the diagrams from memory. In the case of the blind individuals, electroencephalographs were recorded during the resting state,

during an attempt to recall some visual object, and during the recall of some kinesthetic act. Again the changes in EEG were recorded by measurement of the alpha amplitude during each of these phases of the test.

The records taken on normal individuals indicated that those individuals who proved able to reproduce accurately the diagrams which they had been shown during the test were individuals who demonstrated a relatively non-rhythmical type of electrical activity from the occipital areas during the non-stimulated state. Those individuals who were inaccurate in this reproduction were found in general to have a higher alpha potential in the resting state.

In all groups, the average reduction of alpha activity during the actual presentation of a picture was 54.5 per cent. In these same individuals, the attempted recall of the previously presented picture resulted in a 31 per cent reduction in alpha potential.

In respect to the blind individuals, there was suggestive evidence that an attempt to recall a visual image caused a greater potential change in the occipital region in the blind individuals than did attempts to recall other kinesthetic concepts. The responses among the blind were greater among those who had lost their sight after the age of two years.

This study was inconsistent in one respect, namely, those individuals who showed the poorest ability to recall a presented picture, and who showed the high resting alpha potential, still showed a striking reduction of this potential during actual visual stimulation.

The phenomenon of the alpha response has also been used as a measure of the individual's degree of contact with his environment. Daumezon & Lairy (56) correlated the responses of the alpha rhythm during stimulation with the "global attitude of the patient in his contact with his environment." They observed that during visual stimulation those individuals who were in poor contact with their environment showed: (a) an abnormally long latency of the arrest reaction following this stimulus, but later a momentary reinforcement of the alpha activity; (b) a reappearance of the alpha activity on a flat background as a response to certain stimuli; (c) an increase in the threshold of reactivity; and (d) a certain lability of waking rhythms which alternated with sleep potentials. The study of psychotic patients has been carried somewhat further by Bloom (57).

Bloom studied the electroencephalogram and the subjective responses of 11 normal individuals, 9 with organic brain damage, and 12 with schizophrenia. These individuals were subjected to photic stimulation at approximately their alpha frequency. The responses of the electroencephalogram were recorded, and in addition, the individuals were required to report the subjective experiences, and the image formation which occurred during the stimulation.

In this group, it was observed that blocking of the alpha activity occurred with less regularity in both of the patient groups when these were compared with normal individuals. In the schizophrenics, rather than blocking of the alpha, driving was a more prominent response.

The decreased responsiveness of both of the patient groups was also evident in respect to the productivity of their subjective sensation and images. In this respect the greatest response was obtained from the normal, and the least from the organically damaged individuals.

In summarizing the results of the studies reported above in this review, one can record satisfying progress in a number of important areas. There has been reported additional evidence regarding the anatomical connections, and the functional results of ablation and stimulation of deep-lying structures of the reticular formation and of the rhinencephalon—structures whose vital functions have previously been but dimly understood. Further accurate information of the higher functions of the isocortex has been accumulated through impressive collections of data from case reports and from meticulous analysis of intellectual defect. Finally, new data regarding the electrical concomitants of reflex activity in these higher centers are bringing us closer to an understanding of the mechanism underlying the still undefined functions of attention, consciousness, learning, and memory.

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PHYSIOLOGY OF VISION¹

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This review covers the period from June 1954 to May 1957. Lack of space makes it necessary to exclude parts of the subject: the optics of the eye, ocular movements, the mechanism and reflexes of accommodation and fixation, and visual psychology are omitted. With these exceptions, it has been my aim, though doubtless not fully achieved, to mention all important and new experimental work on the visual physiology of vertebrates published during the period under review. With theoretical contributions to the subject I have been intentionally much more critical and selective, and only a small fraction of them has been mentioned.

Books and reviews.—Dartnall's "Visual Pigments" (87) is a thorough and balanced account of the chemistry of photolabile pigments which can be extracted from the retina. The third volume of Le Grand's *Optique Physiologique*, covering spatial aspects of vision, including acuity, and related subjects, has appeared (120). Granit's *Receptors and Sensory Perception* (122) contains two important chapters on visual problems. Topics in the biochemistry of retinal pigments have been reviewed by Wald (254, 255), and Morton & Goodwin (173), the relation of quanta to visual thresholds by Pirenne (199), the properties of the peripheral retina by Weale (260), colour vision by Brindley (55), and psychological aspects of vision by Mueller & Berger (179) and by Pickford (198). Wulff (268) has reviewed the physiology of the arthropod eye, which will not be further mentioned here except to direct attention to five important recent papers on electrical activity of the lateral eyes of the marine arachnids *Limulus* and *Tachypleus* (131, 132, 164, 245, 247).

ANATOMY AND HISTOCHEMISTRY OF THE RETINA AND VISUAL PATHWAY

De Robertis (209, 210) and Wolken (265) have added some details to Sjöstrand's (229) earlier description of electron-micrographs of thin sections of rods and cones. The terminal spherules of rods and terminal feet of cones are penetrated by finger-like processes of the bipolar cells, and contain in their presynaptic cytoplasm a large number of vesicles, mostly of diameter 350 to 400 Å which become smaller if the animal (rabbit) is kept in the dark for nine days before it is killed.

The results of applying a large number of histochemical tests to the retinae of various species have been published (100, 101, 102, 110, 135, 206, 227, 263). In the human retina Eichner (102) has found that alkaline phosphatase is largely confined to the walls of retinal and choroidal blood vessels.

¹ The survey of literature pertaining to this review was completed in June, 1957.

Acid phosphatase is abundant in the ganglion cells, in the inner segments of the rods and cones, and in the pigment epithelium. Cholinesterase, as in other vertebrates, occurs mainly in the inner plexiform layer. Succinic dehydrogenase is found in the myoids of cones, the ellipsoids of rods, the ganglion cells, and the feet of Müller's fibres.

A different, and probably more versatile approach to the problem of distribution of enzymes in the retina is that of Lowry, Roberts & Lewis (162), who froze the eyes of rabbits and monkeys, sectioned them tangentially, and measured the amounts of various enzymes in whole sections. They found in both species that malic dehydrogenase and glutamic-aspartic transaminase were very abundant in the inner segments of the rods and cones, that glutamic dehydrogenase was scarce in all layers, and that all the enzymes investigated were scarce in the outer segments of the rods and cones. Lactic dehydrogenase and phosphoglucosomerase were abundant in the outer plexiform layer of the monkey and in all the neural layers of the rabbit.

Hebb (133) has confirmed that the retinae of mammals and birds contain much choline acetylase and the optic nerves little or none. She also finds that this enzyme is abundant in the optic lobes of pigeons and chickens.

Noell (189, 190) has described the effects of azide, iodoacetate, iodate, and oxygen (100 per cent at 760 mm. Hg for 40 hr.) on the histological structure of the retina, and correlated them with the effects of the first three of these on the electroretinogram.

Wolter (266, 267) reports two observations which support the view that some of the fibres of the human optic nerve have their cell bodies in the brain, and hence are likely to be efferent in function. In the optic nerves of two patients whose eyes had been removed 11 and 16 years before, numerous structures which appeared to be nerve fibres were present; and in a patient with diabetic retinopathy, "typische Cajalsche Endkolben" were found on the stumps of nerve fibres not only running towards the optic disc, but also away from it.

Tereza (243) describes, with photographs, binucleate cells in the ganglion cell layer of six-month-old mink which are believed to be stages in the amitotic division of differentiated ganglion cells.

Descriptive studies of the optic tract of the cat (30), and the striate cortex of the cat (225, 226) and of several species of monkey (224) have been published. A notable observation of Sholl (225) on the cat's cortex is that the branches of each fibre of the geniculocalcarine tract occupy a region in the cortex as much as 650 μ in diameter, within which may lie the cell bodies of as many as 5000 neurones.

Chow (72) has carefully repeated the experiment of Le Gros Clark (74) on the effect of exclusion of all light except red upon the cells of the lateral geniculate body in monkeys, and does not confirm Le Gros Clark's findings. In Chow's experiments, no abnormalities could be found in the retina, lateral geniculate body or striate cortex of monkeys kept for eight months in red

light, blue light or darkness. In infant chimpanzees kept in darkness for over a year, however, ganglion cells almost disappear from the retinae (73).

THE PHOTOLABILE PIGMENTS OF THE RETINA AND THEIR DERIVATIVES

Chemical investigations in vitro.—Radding & Wald (204, 205) have studied the capacity of rhodopsin and opsin to combine with hydroxyl and hydrogen ions, and the effects of pH on the stability of both substances. At 3°C. and pH near neutrality, rhodopsin solutions are stable for at least six months as judged by their absorption spectra, but during this time the capacity to regenerate rhodopsin after bleaching on the addition of neoretinene *b* declines. Some change must be occurring in the protein part of the molecule which does not affect the absorption spectrum. In one-hour experiments at 25 to 27°C., solutions of rhodopsin are stable between pH 3.9 and 9.6, but solutions of opsin only in the narrower range from 5.5 to 7.0.

Barer & Sidman (18) have briefly reported that clear stable solutions of rhodopsin free from detergents can be obtained by disrupting suspensions of rod outer segments with ultrasonic vibrations.

The structures of all the six known geometrical isomers of retinene are now established (56, 192, 193, 207, 208). Numbering the double bonds of the side-chain as 7, 9, 11 and 13 in order from the β -ionone ring, neoretinene *b*, the one which combines with opsin to form rhodopsin, is 11-*cis*. Isoretinene *a* is 9-*cis*, neoretinene *a* 13-*cis*, isoretinene *b* 9, 13-*dicis*, and neoretinene *c* 11, 13-*dicis*.

Hubbard (137) has shown that saline extracts of ox retina or frog pigment epithelium contain an enzyme which catalyses the conversion in the dark of neoretinene *b* to an equilibrium mixture of 95 per cent all-*trans* retinene and 5 per cent neoretinene *b*. The enzyme also increases by up to five times the rate of conversion of all-*trans* retinene to neoretinene *b* by light. It has no action on vitamins A or on isoretinene *a* or neoretinene *a*.

Regeneration of rhodopsin in homogenates of bleached frog and toad retinae (with pigment epithelium) is unaffected by pilocarpine and related drugs (197), despite several earlier reports that they accelerate regeneration *in vivo*.

Pitt, *et al.* (174, 202) have investigated the properties of the Schiff's base formed by combination of retinene with methylamine, and used them to provide evidence on the nature of indicator yellow.

Iodopsin, the photolabile pigment present, in addition to rhodopsin, in chicken retinae has been studied in detail by Wald, Brown & Smith (256). Retinene₁ (or vitamin A₁ if the alcohol dehydrogenase system is present) is liberated when it is bleached. Addition of neoretinene₁ *b* to bleached retinal extract rapidly yields a substance indistinguishable from iodopsin. Rhodopsin also is formed if excess neoretinene *b* is added, but 527 times more slowly. Addition of all -*trans* retinene or neoretinene *a* causes no regeneration.

Isoretinene *a* yields a new photolabile pigment, "iso-iodopsin," with absorption maximum at 510 μ . It appears that iodopsin and rhodopsin differ in their specific proteins, "photopsin" and "scotopsin," and that isiodopsin is the photopsin-analogue of iso-rhodopsin [in Hubbard & Wald's (138) sense of that name, not in the earlier sense of Collins & Morton (79)], as iodopsin is of rhodopsin.

Other photolabile pigments have been extracted from the retinæ of the lamprey *Petromyzon marinus* (82), the fish *Alburnus lucidus* (85), *Gillichthis mirabilis* (184), *Salmo irideus* (43) and *Conger vulgaris* (257), the toad *Xenopus laevis* (86, 253), and the gecko *Phyllurus milii* (83). Photosensitivity has also been detected in digitonin extracts of separated cones of the tortoise *Geoclemys reevesii* (150), but the difference spectrum obtained is unlikely to be due to a single pigment, and has not been satisfactorily analysed.

Investigation of photolabile pigments in the intact retina.—Campbell & Rushton (68, 221) and Rushton (218, 219) have developed optical methods for measuring rhodopsin in the living human retina. The optical density of rhodopsin in the rods is not less, and probably not much more, than 0.15. Regeneration after bleaching follows an exponential curve with a half-return period of seven minutes. The difference spectrum of the extrafoveal retina on bleaching is similar to that of rhodopsin in solution, but displaced 5 to 10 μ towards the red. This close agreement is obtained only if bleaching lasts at least five minutes. For briefer bleaches, a discrepancy is found which can be explained if bleaching *in vivo* produces an unstable orange intermediate product. The action spectrum for bleaching *in vivo* agrees exactly with the scotopic sensitivity curve of the eye. The photosensitivity, when corrected for transmission losses and molecular orientation, is about 1.7 times that of rhodopsin in solution.

Application of similar methods to the foveal retina (216, 217, 220) has proved that at least two photolabile pigments different from rhodopsin are present. These are presumably cone pigments. In protanopes, only one foveal pigment could be detected. Regeneration is much faster than for rhodopsin: it is half complete in 90 sec., and complete in about six min.

Hagins (128) has investigated, in the freshly excised albino rabbit eye, the dark reaction which follows the action on the retina of a very brief bright flash of white light. The fraction of incident light at 400 μ reflected by the fundus oculi is not instantaneously affected by the flash, but rises exponentially with a half-time of 20 msec. at 12°C. For a measuring light of wavelength 486 μ , the fraction reflected is instantaneously slightly raised by the flash, then falls exponentially with the same half-time. It is suggested that this dark reaction may be the breakdown of meta-rhodopsin, although its speed is very much greater than that of the breakdown in aqueous (digitonin) solution of orange-coloured intermediates probably identical with meta-rhodopsin described by Wald and by Lythgoe & Quilliam in 1938. The greatest amount of rhodopsin which, in the rabbit's retina, can be bleached by a single bright flash of less than 1 msec. duration, the rapid dark reaction

being allowed to proceed to completion, is approximately 50 per cent of the amount present before the flash [Hagins (127)].

In the anaesthetized albino rat, Lewis (159) has found that rhodopsin regenerates at the same rate in darkness as when the retina is illuminated: it appears that the photoisomerization of all-trans retinene is not the limiting factor in regeneration.

Weale (258, 259) has measured the effects of illumination on the reflecting properties of the guinea-pig's and squirrel's fundus oculi, using visual methods likely to be less accurate than the refined photoelectric techniques of Rushton and Hagins. In the guinea-pig he obtained evidence suggesting that four different photolabile substances may be present.

Other methods of investigating retinal pigments.—Denton (89, 90, 91) has measured the dichroism of the rods in the edge of folded retinæ of *Salamandra maculosa* and *Xenopus laevis*. Polarised light, incident from the side, is much more heavily absorbed if its electric vector is perpendicular to the axes of the rods than if it is parallel to them. The curves of amount of dichroism against wavelength for the unbleached state are similar to the absorption spectra of rhodopsin (*Salamandra*), or Dartnall's (86) visual pigment 523 (*Xenopus*).

Immediately after bleaching in white light, the strong dichroism of the unbleached rods in the green disappears, and is replaced by a dichroism in the same direction in the violet and near ultraviolet. During the half hour following bleaching, this dichroism disappears in *Salamandra*, but in *Xenopus* it reverses, so that near ultraviolet light polarised along the axes of the rods is preferentially absorbed. The retinæ of several deep sea fish, when tested by this technique, are found to contain pigments with maximum dichroism around 480 m μ , the retinæ appearing to the naked eye golden-yellow in colour (92, 93).

Denton & Wyllie (94) have measured by a photographic method the difference spectrum of single rods in a frog's retina mounted flat and illuminated from its anterior surface. The mean change of density on bleaching was 0.66 at 520 m μ for the pink rods. This implies a total density of rhodopsin at 500 m μ of about 0.82. The green rods, which occupy about 8 per cent of the area of the frog's retina, become on bleaching more transparent in the blue (400 to 444 m μ), but less transparent in the green (490 to 520 m μ).

Dobrowolski, Johnson & Tansley (95) have developed a method for measuring the difference spectrum of single isolated rods.

The amount of rhodopsin extractable from the frog's retina has been reported as greatly decreased by hypophysectomy (240), and the rate of its regeneration as augmented by injections of L-methionine (194).

HUMAN SENSORY EXPERIMENTS

Colour matching.—The colour matching properties of the normal trichromatic eye have been re-examined very thoroughly by Stiles (236, 238), the colour mixture functions being measured directly, and not derived by

multiplying the unit co-ordinates by the luminosity function. A number of interesting subsidiary observations were made, especially on the effects of the intensity of the fields on colour matches, both foveal (2°) and mainly extra-foveal (10° centered on the fovea). A similar but less complete investigation has been made by Friedrich (112, 113).

Brindley (47) has found that in the extreme red, the locus of the spectrum in the chromaticity diagram returns along itself: to each wave-length greater than about $700\text{ m}\mu$ there corresponds a wavelength less than $700\text{ m}\mu$ which is of the same chromaticity.

A theoretical explanation of this, and also of the shape of the scotopic luminosity curve, in terms of the distribution of internal energies of pigment molecules and the condition that a molecule shall be able to absorb a quantum of given energy, has been put forward by Lewis (160).

Trezona (249, 250) has examined, on a $1^\circ 20'$ field, the validity of Grassmann's third law of colour mixture, that lights which match can be substituted for one another as constituents of other colour matches. The observed deviations from the law are so small that it is doubtful whether they can be taken as contradicting physiological hypotheses which predict that the law should hold.

Bongard & Smirnov (36) report that for a 0.5 to 2° field at 1 to 2° from the fixation point, three degrees of freedom are in some circumstances insufficient for colour matching: four are required. This does not agree well with earlier published work, but deserves to be re-examined. Stiles (237) has discussed quantitatively the way in which rods may contribute to extra-foveal colour discrimination, on the assumption, contrary to Bongard & Smirnov, but in agreement with other experimenters, that this is trichromatic.

Brindley (44, 48) has studied the way in which a monocular foveal colour match is disturbed by adaptation to very bright lights. The results are consistent with three kinds of foveal receptors, each containing a single-receptive pigment, if the pigment of the red receptors has the surprisingly high density of 0.98 . Alternative (and less plausible) explanations involve either more than three receptive pigments or a photolabile screening pigment in front of one class of receptors.

Grützner (126) reports that colour discrimination for $20'$ foveal fields is not tritanopic if light-adaptation is preserved by providing an extensive white background of the same brightness as the test fields.

In a very thorough investigation (57) of the errors made in colour matches on a fixed reference colour, the distribution of settings has been found to be Gaussian for each of the instrument primaries, and along each of the axes of the colour-discrimination ellipsoid.

Two theorems of importance in making physiological inferences from the results of colour-matching experiments are proved by Brindley (53).

Absolute and incremental threshold experiments relevant to colour vision.—Stiles (235) has extended his earlier work on foveal increment thresholds to a

group of five subjects. The new measurements make it necessary to postulate two new mechanisms in addition to the three of the original analysis, the threshold of each mechanism depending only on the extent to which it is itself stimulated by the background, independently of the activity of the others. One of the new mechanisms (π_3) has almost the same spectral sensitivity as the original "blue" mechanism (π_1), but differs in that it determines threshold only when the background is very bright.

Aguilar & Stiles (2) have investigated the Weber fraction $\Delta I/I$ for extrafoveal increment thresholds of green on red which are determined (as is proved by their lack of directional selectivity) by rods. They find that the Weber fraction of the rods, which is approximately constant over the range 0.01 to 150 scotopic trolands, increases steeply at higher luminances, so that above about 1000 scotopic trolands, even with background and test wavelengths most favourably chosen for the rods, it is always cones which determine increment thresholds.

Boynton (38) has measured increment thresholds for test flashes delivered only 0.05 sec. after switching on the adapting field, which was exposed for only 0.56 sec. Four adapting fields and a wide range of test wavelengths were investigated.

Auerbach & Wald (15, 16) have found that the cone part of the extrafoveal dark-adaptation curve can be separated, by varying the adapting and test wavelengths, into a number of distinct branches probably attributable to different classes of cones. Similar experiments for the fovea are described by Bush (64). Confirmation has been reported (188) of some of the earlier findings of Burch (59) and Brindley (44) on loss of colour discrimination over large parts of the spectrum ("artificial monochromacy") produced by adaptation to very bright lights.

Colour experiments other than those using direct matching or threshold.—The effects of chromatic adaptation on the appearance of colours have been investigated by putting the two halves of the visual fields into different adaptation states (163) and by binocular comparison (149). Departures from Grassmann's fourth law (of additivity of brightness), in the sense that saturated colours appear brighter, and unsaturated less bright, than they should on additivity, have been confirmed (71). Ferguson & Stevens (108) report an interesting "reversed Purkinje shift" at very high luminances: if sodium and mercury light are equalized in apparent brightness at 1 ft-L, then on increasing both proportionally 1000 times or more, the mercury comes to look brighter.

Colour blindness.—Monochromacy (111) and tritanopia and tritanomaly (142) have been well reviewed. Kalmus (147) finds that pedigrees of tritanopia are consistent with autosomal dominant inheritance, with somewhat imperfect manifestation. Baumgardt (23) has found a normal scotopic spectral sensitivity curve in a "cone" monochromat. Schmidt (222) has recorded the anomaloscope settings of 864 male trichromats. In contrast to the earlier work of Nelson, he finds a three-peaked frequency distribution, the

protoanomalous as well as the deuteranomalous being clearly separable from the normal.

Absolute threshold and dark adaptation.—Detailed examinations of the effects of the intensity and duration of preillumination on the course of subsequent dark adaptation have been made for both fovea (143, 144) and periphery (176, 177, 230). The superiority of red light to light of other colours at the same photopic brightness in allowing dark adaptation to proceed has been confirmed (231), but this study, like its predecessors, does not clearly show whether or not lights of the same scotopic brightness always have the same effect in raising scotopic threshold. The variability of dark adaptation curves repeated under identical conditions has been found (175) to be greatest near the beginnings of the "cone" and "rod" parts of the curve, declining during each of the two branches.

Effects of illuminating one eye on the threshold of the other have been affirmed (1, 264) and denied (37, 171). The striking feature of these effects, if they exist, is their smallness; they do not give any clear support to the hypothesis of efferent fibres to the retina capable of modifying its sensitivity reflexly when the other retina is illuminated. Also relevant to the question of whether there may be centrifugal control of the retina is Mertens's finding (167) that the probability of seeing a flash near absolute threshold is only just significantly greater when the subject knows where it is to come, and directs his attention there, than when it is presented at random in any one of four places.

Pirenne (200) has found that when a Landolt's C is presented to extrafoveal retina for only 2.6 msec., the threshold for the gap is about five times higher than if it is isolated on a dark background; but if the Landolt's C is presented for a long time and the eye allowed to scan it, the presence or absence of background does not affect the threshold.

Monjé (172) has explored the raising of foveal threshold by a centrally fixated conditioning stimulus of 9.5' diameter, using a very small test spot placed either within or outside the conditioning field.

Increment thresholds and the nature of visual threshold.—Further reasons for believing that, at absolute threshold, at least four quanta must be absorbed in rods for a flash to be seen have been provided by Pirenne & Marriott (199, 201) and Brindley (45). Barlow (19) suggests that, if a factor of limiting the absolute sensitivity of the eye is the occurrence of "noise" events (perhaps thermal decomposition of rhodopsin molecules) which are indistinguishable from the absorption of quanta in rods, the number of quanta required for threshold is likely to be a good deal more than four. The frequency-of-seeing curves of Hecht, Schlaer & Pirenne are well fitted by the hypothesis that a coincidence of 21 quantum-like events is required for threshold, if on the average nine of these are contributed by "noise" and the rest added by the flash of light. This hypothesis gives reasonable values for the number of false positives obtained in threshold experiments, and explains how different frequency-of-seeing curves can be obtained according to whether the subject

sets himself a high or low standard of certainty that he has seen the flash.

Development by Barlow (20) of the idea that increment thresholds are discriminations of constant fallibility leads to the law $\Delta I \propto (I+x)^{1/2}$, where I is the background intensity and x the light-equivalent of the retinal noise. Data for short flashes of small area (but not those for long flashes or large areas) obey this law up to values of ΔI about 200 times absolute threshold, thereafter deviating in the direction of Weber's law.

Measurements of the intensity-time relation for foveal increment threshold (80), of the least visually detectable time of interruption of foveally fixated point of light at various intensities (58), of the course of increment threshold during adaptation to a steady background (17, 39) and of the effects of after-images on brightness discrimination (214) have been reported.

Directional sensitivity of the retina.—It has been reported that the mydriatic β -phenyl-isopropylamine affects the directional sensitivity of the retina (211), and that lights falling upon the retina from different parts of the pupil do not affect it strictly additively (103).

Spatial aspects of discrimination.—Oliva & Aguilar (191) have measured the two-point discrimination of the dark adapted eye at a large number of points on the retina. Hake & Averbach (129) have found that the discrimination of brightness of two fields at a fixed distance from each other is better if they are separated by an intervening zone of uniform brightness-gradient than if the gap between them is dark. Gregory (124) has investigated the effect on brightness discrimination of varying the relative sizes of the two (contiguous) comparison fields, but keeping the sum of their areas constant. Harms & Aulhorn (130) find that the increment threshold for a test flash of 3' diameter is much higher close to the boundary between two large steady fields of different brightness than within either of them at a distance from the boundary.

Flicker.—Kugelmass & Landis (155) have explored the relation of fusion frequency to the area and luminance of centrally fixated test fields in the range 1.27° to 14.6° diameter. Ettlinger (104) has found that, if a large bright surround is provided, foveal and peripheral fusion frequencies are almost the same. Gebhard, Mowbray & Byham (116) have measured the least detectable difference of flicker frequency in the range 1 to 45 c.p.s. Smythies (232) has described the spatial patterns seen in a very large physically homogeneous flickering field.

Electrical phosphenes.—The fundamental question here is what structures are being stimulated when an electric current passing through or near the eye causes a sensation of light. The only new evidence on this question is that of Brindley (46), who plotted the spatial distribution of phosphenes produced by electrical stimulation with electrodes applied directly to the anaesthetized conjunctiva. In these conditions, the distribution of current within the eye could be inferred with fair accuracy from the passive electrical properties of the system. Its correlation with the distribution of the phosphene indi-

cated that stimulation was due to the component of the current directed radially in the eye, current flowing parallel to the surface of the retina being entirely ineffective. The foveal region departed slightly from this rule. The preferred direction for stimulation at the fovea was what would be expected if here the sensitive structures, instead of being strictly radial, leaned out in all directions from the optic axis. This would agree with their being either bipolar cells, or the parts of the rod and cone cells lying inside the external limiting membrane, or both of these. In no circumstances could stimulation of optic nerve fibres, either inside or outside the eye, be detected.

Howarth (136) has investigated the strength-duration curve for electrical stimulation by single pulses, and strength-interval relation for pairs of short pulses of opposite polarity. Mita, Fujimaki & Takahashi (169) have investigated the relation of the threshold for alternating current to the frequency and to the intensity of simultaneous illumination. Mil'shtein (168) finds that the flicker fusion frequency for electrical phosphenes decreases smoothly as the number of stimuli given is diminished. Gebhard *et al.* (115) have studied frequency discrimination in electrical flicker.

Volumes 62 to 64 of the Tohoku Journal of Experimental Medicine contain seventeen papers on electrical phosphenes by members of Motokawa's department. Assessment of these is probably best deferred until some of the principal results obtained by Motokawa's methods have been independently confirmed; they require the measurement of electrical thresholds with an accuracy far exceeding that which careful workers in other laboratories have hitherto been able to achieve. If they can be reproduced, most of the results are interesting; but the authors have nowhere used them (and it is not clear that they can be used) to provide conclusive evidence against any hypothesis which would be otherwise plausible. The use made of them so far has always been to provide weak support for theories which have stronger (though not always strong) foundation in data of other kinds.

ELECTRICAL ACTIVITY OF THE RETINA

Origin of the electroretinogram.—Noell (189) has found that in rabbits iodate, which is found histologically to damage principally the pigment epithelium, abolishes the *c*-wave of the electroretinogram without affecting the *a*- and *b*-waves. This suggests that the pigment epithelium is necessary for the production of a *c*-wave, and may perhaps be the structure which generates it; though, at least in the frog, the pigment in which light must be absorbed to produce a *c*-wave is rhodopsin, as Granit & Munsterhjelm (123) showed long ago by measuring its spectral sensitivity.

Brindley (49 to 52, 54) has obtained evidence that the *a*-, *b*- and *d*-waves of the frog's electroretinogram are wholly or almost wholly generated by the rods and cones. In favourable circumstances a typical normal electroretinogram may be recorded with a microelectrode at all points within a retina in front of a structure of high electrical resistance and capacity which is almost certainly the external limiting membrane. As the electrode crosses the ex-

ternal limiting membrane, the response suddenly disappears, a fact indicating that all the generating structures are capable of carrying current across the external limiting membrane. This argument is not invalidated by the finding, in other, and, it is thought, functionally more normal preparations, of a complex sequence of responses to illumination differing from the electroretinogram which can be recorded from layers of the retina in front of the external limiting membrane, since the possibility of these complex responses being absent at all points of a retina without any abnormality in the electroretinogram indicates that they make no contribution to it. The exact spatial additivity found in the electroretinogram (50, 54), indicating that the generating structures do not interact with each other, provides further support for the hypothesis of origin from receptors only.

Effect on the electroretinogram of the temporal pattern of stimulation.—The Bunsen-Roscoe law holds for the electroretinogram up to about 0.1 sec. in man (3, 145) and 0.3 sec. in the frog (151) in the dark adapted state, the maximum duration decreasing in light adaptation. The effects of pairs of stimuli closely following each other (5, 183, 262), and of linearly rising (212, 213) and exponentially falling (148) stimuli have been described. For flickering stimuli of 110° diameter in man, the fusion frequency is slightly higher for the electroretinogram than for sensation (27). At 40 to 60 flashes per second, the responses may be alternately large and small (27).

Electroretinographic sensitivity during dark adaptation.—Dark adaptation curves using a constant height of *b*-wave as criterion have been published for man (26) and squirrel (241). The human curve follows that of visual threshold closely, with a sudden increase of gradient, presumably attributable to the change from the photopic to the scotopic mechanism, at about ten minutes. Lights of constant intensity-time product have constant adapting effects on the frog's electroretinogram certainly up to 100 secs. and probably up to 1000 (151).

Effects of chemical agents and ischaemia on the electroretinogram.—The effects of potassium (84, 114), ammonium, lithium, calcium, aspartic acid (114), methyl and ethyl alcohols, formaldehyde, formic acid (203), epinephrine (215), iodate, iodoacetate (189), veratrine, pentobarbital, urethane (84), dithizone (88, 261), alloxan, neotetrazolium (88), γ -hexachlorocyclohexane (223, 252), brucine, and acetylcholine (25) have been described. Oxygen has a very beneficial effect on the survival of the electroretinogram (e.r.g.) in excised frogs' eyes (152). Hyperventilation increases the *b*-wave and decreases the *a*-wave of the human e.r.g. (4). Hypoglycaemia in rabbits decreases the size of the *b*-wave and causes the e.r.g. to fail more rapidly in ischaemia and recover less completely after it (195, 196). In centrifuged human subjects, an acceleration causing loss of peripheral and central vision (roughly 5.5 g) has only a slight and inconstant effect on the e.r.g. (158). Light adaptation applied within four minutes of the beginning of a ten-minute period of ischaemia in the rabbit's eye (while the retina is still electrically responsive) augments the *a*- and *c*-waves which can be obtained

immediately after restoring the circulation; but if the light-adaptation is applied in the latter six minutes of the ischaemic period, when the retina is electrically unresponsive, it has no effect on the *a*- and *c*-waves subsequently obtained (9).

Spectral sensitivity of the e.r.g.—A Purkinje shift in the electroretinographic spectral sensitivity curve is absent in the squirrel (7) and souslik (8), and present in man (13, 27) and the chicken (14). The spectral sensitivity for electrical responses of the human eye to light flickering at 20 per sec. resembles that of photopic vision (11) and is unaffected by superimposing the flickering test light on steady backgrounds of various wavelengths (12). Responses to flicker at 4 per sec. are not simple in shape, and their spectral sensitivity is not stable to coloured backgrounds (12). This could be due to the persistence of some rod activity. In opened excised frogs' eyes, both light and dark adapted, Forbes, Burleigh & Neyland (109) find that sudden replacement of uniform illumination of the whole retina with monochromatic light of one wavelength by similar illumination with light of a different wavelength causes a small (about 30 μ V) electrical response which cannot be abolished by adjusting the relative intensities of the two lights. In the turtles *Pseudemys scripta elegans* and *Chrysemys picta*, similar but larger responses are obtained. In man, it seems that the response to replacement can always be abolished by a suitable adjustment of relative intensities, at least in the range tested by Biersdorf & Armington (28). Goto & Toida (117, 118, 244), examining closely the off-responses given by excised frogs' eyes, find that complexities of shape occur which differ consistently for different stimulating wavelengths.

Development of the e.r.g. In normal kittens, the e.r.g. first appears about one week after birth, and reaches adult size and form at about nine weeks. In kittens reared in darkness, it does not appear until about three weeks, but is the same as in light-reared by the sixth week (269).

Slow electrical activity recorded with microelectrodes from within the retina.—From freshly excised frogs' eyes complex-electrical activity on illumination, comparable in time-course to the e.r.g., but apparently making little or no contribution to it, can be recorded from the inner nuclear, outer synaptic and outer nuclear layers. These complex intraretinal responses have been described, both for diffuse and for various patterns of focal illumination, by Tomita & Torihama (248), Brindley (51, 52) and Müller-Limmroth & Güth (182). It is probable that both horizontal and bipolar cells contribute in generating them. No components likely to be produced by amacrine or ganglion cells have been described.

In the South American fish *Mugil*, Svaetichin (239) has recorded with microelectrodes monophasic responses to illumination which, for some positions of the electrodes, are negative-going for light of short wavelengths and positive-going for long wavelengths. At other electrode positions, negative-going responses are obtained for all wavelengths, or negative-going for long and positive-going for short. Svaetichin suggests that the responses which

reverse their polarity with wavelength may be intracellularly recorded responses of twin cones. Motokawa, Oikawa & Tasaki (178) have reported very similar findings in another fish, *Cyprinus carpio*. However, Tomita (246) has provided evidence that when a microelectrode records, from a fish retina, responses very similar to those described by Svaetichin, its tip is in front of the receptors, and probably extracellular.

Spike potentials from retinal ganglion cells.—Barlow, FitzHugh & Kuffler (21, 22, 154) find that all the ganglion cells in the cat's retina from which spikes can be obtained show maintained activity in darkness or in steady light. The relation of the frequency of the maintained discharge to the intensity of light varies much from one cell to another. The intervals between impulses do not show the exponential distribution characteristic of uncorrelated events.

In dark adaptation, which proceeds about four times more slowly than in man, the cat's ganglion cells increase their sensitivity about 10,000 times. All ganglion cells show a Purkinje shift, so that presumably all are connected to both rods and cones. In the dark adapted state, the inhibitory fringe of the receptive field, which Kuffler (153) earlier found to give off-responses in those units for which the centre gave on-responses, and on in those for which the centre gave off, disappears. This change of organization occurs only when dark adaptation is nearly complete. It is not linked to the Purkinje shift, and does not depend on the change from cones to rods.

Grüsser & Creutzfeldt (125) find that for uniform flickering illumination of the cat's retina, each ganglion cell gives maximum impulse frequency at a flicker frequency somewhat below that of fusion. Bykov has described for the frog (65) and cat (66) the effects on the latent period of ganglion cells of varying the frequency and intensity of regularly repeated stimuli.

Dodt & Elenius (99) have measured the spectral sensitivity of ganglion cells in the rabbit's retina. In the dark adapted state it follows the absorption spectrum of rhodopsin, except for a variable additional peak in the blue around 460 m μ . On light adaptation, the wavelength of maximum absorption does not change, but sensitivity to red and blue become relatively a little greater. Dodt (98) has also measured the spectral sensitivity for the least and the greatest amount of light which, when interrupted 21 times per sec., will produce a flicker response in rabbit ganglion cells. Bongard (35) finds that sudden replacement of green light by red on the frog's retina always produces a discharge of spikes in the optic nerve, however the relative intensities are adjusted; but replacement of green by a mixture of red and blue can, with suitable adjustment of the relative intensities, be carried out without provoking any discharge in the nerve.

X-rays resemble light in producing both on- and off-discharges from the frog's ganglion cells, the pattern of response of a given cell being the same for both stimuli (161).

Resting potential of the eye.—The "R membrane" (probably the external limiting membrane) has in the opened excised frog's eye a steady difference

of potential across it of from 10 to 30 mV (49). This could be the main component of the eye's resting potential. In the rabbit, the resting potential, as detected by the electro-oculogram (change in the potential difference between electrodes on the orbital margins on rotating the eye), is only slightly diminished by doses of iodoacetate sufficient to abolish the e.r.g. (134). Iodate abolishes the resting potential in doses which have no effect on the *b*-wave of the e.r.g. (134). In both frog (181) and rabbit (134) the resting potential is increased by light adaptation.

Electrical evidence for the existence of efferent nerve fibres to the retina.—Granit (121) has found that stimulation of points in the reticular formation of cats often potentiates or inhibits the response to light of retinal ganglion cells which are not antidromically excited. Though it is clear that the observed effects are not due to stimulation of the axon of the ganglion cell from which spikes are being recorded, the possibility does not seem to be excluded that they may be due to stimulation of axons of other ganglion cells, the fibres concerned being normally afferent, and only showing efferent effects under the special conditions of the experiment. The same consideration could perhaps apply to Dodt's (97) finding that stimulation of the optic tract in the rabbit causes an antidromic spike recorded by a microelectrode on the anterior surface of the contralateral retina, followed after 7 to 25 msec. by a delayed spike highly sensitive to the state of adaptation, to mephenesin, and to strychnine, none of which affect the antidromic spike. If, however, as both authors suggest, the fibres concerned in these phenomena are normally efferent, it becomes very interesting to speculate on their function in the intact animal.

Müller-Limmroth (180) has found that, on illumination of one eye in the guinea-pig, a slow electrical response can be recorded with large electrodes from the other eye, from which the anterior half, including the iris, has been cut away. This does not appear to be physical conduction of the e.r.g., from one eye to the other, since it is abolished if both lateral geniculate bodies are removed. It may be related to the phenomena described by Granit and Dodt.

ELECTRICAL ACTIVITY OF THE HIGHER PARTS OF THE VISUAL PATHWAY

Velocity of conduction in fibres of the optic nerve.—This has been investigated in the cat by P. O. Bishop, Jeremy & Lance (32), G. H. Bishop & Clare (30), and Chang (69). There is an apparent disagreement between these authors which needs examining. P. O. Bishop, stimulating the optic tract and recording from the optic nerve close to the eye, finds two waves in the monophasically recorded response, corresponding to mean conduction velocities of 34 and 18 m. per sec. for stimulation of the contralateral tract, and 35 and 21 for the ipsilateral. The spread of velocities within each group is such that for the fastest fibres (estimated from the foot of the first wave) it reaches about 70 m. per sec. G. H. Bishop & Clare, stimulating the nerve

and recording from the opposite lateral geniculate body, find two presynaptic waves corresponding to velocities of 40 to 50 and 15 to 25 m. per sec., in fair agreement with P. O. Bishop. By examination of postsynaptic responses they infer the existence of two groups of slower fibres, with velocities of 6 and 3.5 m. per sec. Neither they nor the other experimenters have been able to detect these slow fibres by direct methods. This failure does not disprove their existence, but the arguments by which G. H. Bishop & Clare identify them and estimate their conduction velocities are not wholly convincing. Chang (69), using nearly the same technique as P. O. Bishop, finds that in certain conditions three waves can be distinguished, corresponding to velocities of about 70, 30 and 17 m. per sec. Three waves are conspicuously distinct only if both optic tracts are stimulated simultaneously, when the interpretation is difficult owing to uncertainty as to the relative length of crossed and uncrossed fibres; but with favourable placing of the stimulating electrode they can just be distinguished on stimulation of the contralateral tract alone.

Whether the distribution of velocities, excluding those of Bishop & Clare's hypothetical very slow fibres, is in fact bimodal or trimodal is not of great importance in interpreting experiments on the lateral geniculate body or visual cortex, since in many of these experiments the situation has here been simplified by using shocks weak enough to stimulate only the fastest group.

Dodt (96), stimulating the cat's lateral geniculate body and recording action potentials from the surface of the contralateral retina, finds a bimodal distribution of velocities for the myelinated extraocular parts of the fibres. The unmyelinated intraocular parts of the fibres conduct at mean velocities of 2.86 m. per sec. for the fast group and 1.7 m. per sec. for the slow.

Lateral geniculate body.—P. O. Bishop & McLeod (34) find that, in stimulating the optic nerve and recording from the optic tract, the spike due to the fast fibres is about 90 per cent of maximal at threshold for the slow fibres. Recording from the lateral geniculate body and keeping the stimulus below threshold for the slow fibres, the presynaptic and postsynaptic spikes maintain a constant ratio of amplitudes as the strength of stimulus is changed. This suggests that very little spatial summation is required to discharge neurones of the lateral geniculate body. P. O. Bishop & Evans (31), studying the recovery of excitability in the geniculate synapses, find that, when a test shock follows a conditioning shock by a sufficient interval to produce a presynaptic spike, it always produces a postsynaptic spike also. The absolute refractory period of the synapse is thus probably no longer than that of the optic nerve fibres. The synaptic delay, which is about 0.3 msec., is not substantially affected by a previous conditioning shock. Hughes, Evarts & Marshall (105, 106, 139) have investigated the effects on conduction across the geniculate synapses of previous stimulation of the optic nerve at 500 per sec. This causes the postsynaptic response for shocks of constant strength to be depressed for about two seconds (first subnormality), and then enhanced for about a minute (posttetanic potentiation). In cats anaesthetized with

pentobarbital, but not in unanaesthetized decerebrate preparations, post-tetanic potentiation is followed by a second period of subnormality lasting at least ten minutes.

Recording with microelectrodes of about 0.3μ tip diameter from within the lateral geniculate body, Tasaki, Polley & Orrego (242) distinguish four types of extracellularly detectable response to electrical stimulation to the optic nerve. They identify these as the responses of presynaptic axon, postsynaptic axon, cell body, and dendrites. The last three of these often, and the first never, respond multiply to a single shock, in agreement with the earlier findings of Bishop, Jeremy & McLeod (33) with larger microelectrodes.

Both spikes (77, 78) and slow electrical changes (140, 157) have been recorded from the cat's lateral geniculate body in response to illuminating the eyes. Cohn (77) finds that when the contralateral eye is illuminated spikes can be obtained only from layers A and B, and when the ipsilateral is illuminated, only from the intermediate layer A_1 . This agrees with anatomical evidence on the distribution of transsynaptic degeneration after removal of one eye (77, 228); but there is no clear evidence on whether Cohn's spikes are pre- or postsynaptic.

Vastola (251) finds that repetitive stimulation of the cat's optic nerve produces, for about five seconds after the end of stimulation, a potential gradient in the lateral geniculate body of the polarity that would be expected if the cell bodies of the postsynaptic neurones became negative to their axons.

Tectum.—Electrical activity in response to light or to stimulation of the optic nerve has been described in the tectum of fish (60, 61, 63), amphibia (6, 60, 170) and birds (60, 81).

Visual cortex.—Four attempts have been made (29, 42, 70, 166) to analyse the sequence of four surface-positive waves followed by a slow surface-negative wave by which the cat's visual cortex responds to an afferent volley initiated at any point in the visual pathway. The four surface-positive waves will here be referred to as 1, 2, 3, and 4, in order of their occurrence. Chang & Kaada (70) conclude that 1, 2, and 3 are presynaptic, and separated by differences in extracortical conduction time; only 4 is postsynaptic. G. H. Bishop & Clare (29) assign only 1 to the presynaptic fibres, and 2, 3 and 4 to cortical neurones. According to Malis & Kruger (166), 1 and 2 are presynaptic and separated by differences in extracortical conduction time, and 3 and 4 are postsynaptic. Finally Bremer & Stoupe (42) suggest that 1, 2, and 3 are presynaptic, but separated by differences in intracortical conduction time; 4 is postsynaptic.

The principal relevant observations are the following. All components of the response increase proportionally as the strength of electrical stimulus to the optic nerve is increased (70). Strychnine applied locally to the cortex greatly increases 4 without having any effect on 1, 2 or 3 (42, 70, 119). Illumination of the eyes potentiates all components of the response, 3 and 4 being slightly more affected than 1 and 2 (42, 166); but electrical stimulation

of the contralateral visual cortex, the corpus callosum being intact, potentiates 4 without effect on 1, 2 or 3 (42). The responses to stimulation of the optic nerve, lateral geniculate body, and optic radiation differ much in latency, but very little in the spacing of the waves (42, 166). Observations on the response recorded from the underlying white matter after removal of the visual cortex are conflicting (42, 166).

All these findings are consistent with the hypothesis of Bremer & Stoupel, and some of them are difficult to reconcile with the other hypotheses; but the question cannot be considered finally decided.

Bremer (40, 41) finds that a single electrical stimulus to the visual cortex causes, if the corpus callosum is intact, a response in the visual cortex of the opposite side extremely similar to that produced by stimulation of the optic nerve or optic radiation.

The pattern of impulses produced in single neurones of the visual cortex on illumination of the eye has been described by Jung & Baumgartner (24, 146) and by Cohn (78). Slow electrical responses of the visual cortex to illumination of the eye have been described for the cat (62, 156, 165) and for man (67). Photically and electrically induced responses have also been studied in cortex under the influence of strychnine (10, 42, 70, 75, 76, 119) pentylene-tetrazol (Metrazol) (140) and lysergic acid diethylamide (107).

Visually-provoked electrical activity outside the main visual pathway.—Ingvar & Hunter (141) have detected electrical activity in response to brief flashes falling upon the eyes from a large variety of sites in the brain-stem of cats. This activity is not prevented by bilateral ablation of the visual cortical projection areas and some adjacent cortex, carried out some weeks before the experiment.

HIGHER PARTS OF THE VISUAL PATHWAY STUDIED BY METHODS OTHER THAN ELECTRICAL

The discrimination of visual patterns by cats is not much impaired by the insertion into the visual cortex of about 30 pieces of tantalum wire or about 15 plates of mica on each side (Sperry, Miner & Myers, 233, 234). Cats, which have first had the optic chiasma sectioned mid-sagittally and then been trained to make a visual pattern discrimination with one eye, are found to perform almost equally well if subsequently tested with the other eye (Myers, 185). This interocular transfer is lost if the whole corpus callosum or its posterior half is destroyed, but is unaffected by partial destruction of the corpus callosum provided that the posterior 25 to 30 per cent remains intact (186, 187).

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